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Reproductive biology of *Sphaeralcea* species with ornamental interest

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Key words: Combining ability, Malvaceae, mating system, pollen viability, stigma receptivity.

Abstract: The genus *Sphaeralcea* belongs to the Malvaceae family and has native species from South America. Their attractive morphological characteristics with ornamental value have not yet been explored. The objective of this work was to know the viability of pollen, stigma receptivity, type of pollination and combining ability of four *Sphaeralcea* species (*S. australis*, *S. bonariensis*, *S. crispa* and *S. mendocina*), with the aim to develop new ornamental varieties. Fructification, fertility, seed germination and survival seedlings on intraspecific and reciprocal interspecific offspring were assessed. The highest values of stigma receptivity and pollen viability were obtained at 2:00 PM for the four species. *S. mendocina* also showed high values of pollen viability at 4:00 PM .The species proved to be self-incompatible and allogamous, with different degrees of reproductive compatibility. The interspecific crosses of *S. mendocina* and the intraspecific of *S. crispa* did not produce descendants. The crosses between *S. australis* and *S. bonariensis* as maternal parent presented the best combining ability with good fruit production, seed germination and survival. This research provides useful information for the formulation and implementation of breeding strategies, to improve pollination efficiency, and to breed new *Sphaeralcea* varieties with ornamental potential.

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1. Introduction

The Malvaceae family is worldwide distributed in regions with temperate and warm climate. In South America, are represented by 63 genus and 533 species of herbs, shrubs and trees, from these 315 are native, 202 endemic and 16 exotics (Zuloaga *et al*., 2019). Some species are economically important, like various *Gossypium* species, including cotton. Others have medicinal properties (Martínez and Barboza, 2010) and ornamental interest (Krapovickas, 2003; Gutiérrez *et al.*, 2021). Some genera of Malvaceae with ornamental potential are *Pavonia*, *Lecanophora*,

Modiolastrum, *Rynchosida* and *Sphaeralcea* (Ponce *et al*., 2006; Torres *et al*., 2008; Masini and Rovere, 2015; Gutierrez *et al*., 2021). In Argentina, the *Sphaerlacea* genus have native and herbaceous species with attractive characteristics for ornamental cultivation, such as *S. australis*, *S. crispa*, *S. mendocina* and *S. bonariensis* (Sriladda *et al*., 2012; Gutierrez *et al*., 2021). They are adapted to semi-arid conditions, and have tolerance to water stress, high and low temperatures and high insolation, which make them good candidates for breeding programs in urban ecosystems adapted to extreme weather conditions and for sustainable landscaping. Moreover, the use of native germplasm in breeding programs contributes to the conservation of biodiversity (Masini and Rovere, 2015). In general, native plants make more efficient use of environmental factors such as water and other climate factors, as well as edaphic and biological variables, which result in a lower maintenance demand and in a good performance under the restrictive local conditions.

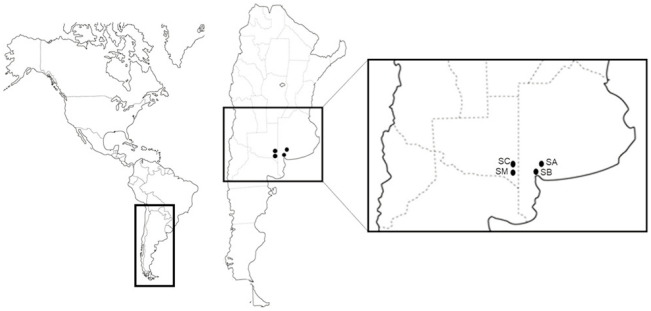
Knowledge of the plants reproductive biology is essential for classical breeding programs, because it allows better orientation and planning of the crosses. In the case of the species under study, their reproductive biology is unknown. This knowledge is essential to be used in pollinations, and to increase the chance of successful fertilization. Some of these aspects are pollen viability, stigma receptivity, pollination type and combining ability since these depend on successful reproduction. Pollen viability and stigma receptivity are aspects that plays an important role in successful hybridizations (Figueiredo *et al.,* 2020). Pollen viability is a measure of male fertility (Liu *et al*., 2021), viable pollen is critical to the process of reproduction and pollen longevity can be affected by temperature and relative humidity (Ren *et al*., 2019). Stigma receptivity is the ability to receive the pollen, therefore it directly affects the plant life cycle, allowing the pollen to adhere, hydrate and germinate (Shivanna and Sawhney, 1997). A detailed knowledge of these features will determine the best moment for pollination, to enable successful controlled pollination in breeding programs. Stigma receptivity is related to the activity of enzymes such as peroxidase, esterase and dehydrogenase (Galen and Plowright, 1987). Receptive stigmas have high enzyme activity, which can occur in different phases of flower development. The observation of the activity of these enzymes can be used to characterize stigma receptivity (Zhang *et al.,* 2021). For the pollination process to occur, the transfer of pollen to the stigma must happen during the period in which the stigma is receptive, otherwise, pollen cannot adhere and germinate.

The aim of this study was to evaluate the reproductive biology of four native species of the genus *Sphaeralcea*, determining pollen viability, timing of stigma receptivity, pollination type and combining ability. The hypothesis we followed was that it is possible to determine aspects of the reproductive biology of the native germplasm of *Sphaeralcea*, with the ultimate objective to develop new ornamental varieties.

2. Materials and Methods

Collection area

The Pampas region is an extensive plain located to the east of Argentina between 31° and 39° south latitude. Aliaga *et al*. (2017) characterized the Pampas general climate, considering rainfall, air temperature, humidity, and wind speed as well as the altitude and the alternation between dry and wet events in the area. Based on these elements, they categorized the Southwest of Buenos Aires and the Southeast of La Pampa as semi-arid region. *Sphaeralceae* seeds were collected between December 2020 and March 2022 in different sites of this semi-arid region (Fig. 1). The sites were characterized by the occurrence of long periods of drought and isolated floods together with windy periods which affect severely the water availability (Aliaga *et al*., 2017).



Species (CODE)	Locality	Province	Location
Sphaeralcea australis (SA)	Tornquist	Buenos Aires	S38°40'76", W62°81'75"
Sphaeralcea bonariensis (SB)	Bahía Blanca	Buenos Aires	S38°45'29", W62°18'47"
Sphaeralcea crispa (SC)	Caleu Caleu	La Pampa	S38°48'29", W63°48'71"
Sphaeralcea mendocina (SM)	Caleu Caleu	La Pampa	S38°55'66", W63°49'76"

Fig. 1 - Geographic distribution and collection sites of studied populations of *Sphaeralcea australis*, *S. crispa*, *S. mendocina* and *S. bonariensis* in central Argentina, South America. For labels see the table.

Plant material and experimental design

The plant material was collected in the indicated area (Fig. 1) as seeds and preserved at 4°C dry with silica gel. The seeds were germinated after pre-germinative treatments to break dormancy (Gutierrez *et al*., 2019). After scarification, the seeds were placed in Petri dishes on filter paper moistened with distiller water in a germination chamber at 20°C (ISTA, 2019) with a 12 h photoperiod previously used in other Malvaceae (Erickson *et al*., 2016; Leperlier *et al*., 2020). Five plants of each *Sphaeralcea* species were grown in pots with commercial substrate (GROWMIX MultiPro®) in the greenhouse of the Center of Renewable Natural Resources from the Semi-Arid Region (CERZOS, CONICET - UNS), under controlled temperature (18-28°C), irrigation and relative humidity (55-85%).

During the day, when the flowers were in anthesis, pollen viability and stigma receptivity were studied at 8:00 h, 10:00 h, 12:00 h in the morning and at 2:00 h, 4:00 h, and 6:00 h in the afternoon.

The same experimental designs were used to evaluate pollen viability and stigma receptivity. Four species (treatments), five plants per species (replications) and three flowers per plant, were used to evaluate a total of 60 anthers and 60 stigmas in a completely randomized experiment.

Stigma receptivity

The Osborn method was used to evaluate the stigma receptivity, based on the reaction of the peroxidase enzyme. The stigma is classified as receptive when placing a drop of hydrogen peroxide at 40% on the flowers stigmas a bubble production is observed (Osborn *et al*., 1988). The reaction of stigma receptivity was examined with a stereomicroscope.

Pollen viability

The estimation of viable pollen was carried out with the Alexander technique (Alexander, 1980). The grain dyed in an intense violet color was taken as viable and the one that was colored green was taken as non-viable. Pollen grain counts were performed in an optical microscope of four random fields per preparation to estimate the percentage of pollen viability (%) = [(*Number of viable pollen grains*/*Number of total pollen grains*) x 100].

Mating system

The experimental trial to test the reproductive system in *Sphaeralcea* genus was based on four experiments and is seen in Table 1. In the greenhouse, the plants of experiments 2, 3, and 4 were isolated with a fine mesh in a cage that excludes potential pollinators. The plants of experiment 1 were used as control and located outside the cage.

For experiment 1, five plants of each species (*S. australis*, *S. crispa*, *S. mendocina* and *S. bonariensis)* were used and three flowers of each plant were marked the day before anthesis. The flowers were allowed to develop normally without any manipulation, as control. The developed fruits were properly identified and covered until harvest.

For experiment 2, five plants of each species were used, and three flowers of each plant were marked the day before anthesis. The fruits were properly identified and covered until fully developed and harvest.

For experiment 3, five plants of each species were used, and three flowers of each plant were marked the day before anthesis. The flowers were allowed to develop normally and without any type of manipulation to evaluate natural self-pollination. The developed fruit were properly identified and covered until harvest.

For experiment 4, inter-specific reciprocal and intra-specific crossing were performed between *S. australis*, *S. crispa*, *S. mendocina* and *S. bonariensis*

Table 1 - Experimental management on *Sphaeralcea australis*, *S. crispa*, *S. mendocina* and *S. bonariensis*. Normal seed set "+" and greatly reduced or zero seed set "-" (modified from Simpson, 2019)

Experimental management	Seed production	
1. Flowers left to develop normally, as control.	+ Fertile	− Infertile
2. Isolated flowers, then self-pollinated by hand.	+ Self-fertile	− Not self-fertile
3. Flowering plants in caged, then left freely.	+ Self-pollinating	− Not self-pollinating
4. Isolated flowers, then emasculated and outcrossed.	+ Outcrossing	− Not outcrossing

(Table 2). Five plants of each species were used, and three flowers for each plant were marked on the day before anthesis. The flower buds were emasculated, the anthers were removed prior to pollen release and reciprocal outcrossing were made once a day during the flowering period. Other flowers were used as male parent. After pollination, the flowers were properly identified and covered until fruit harvest.

The fruiting and fertility results of the four experiments were assessed by counting the seed set in relation to the number of pollinated flowers [Fructification (%) = (*number of fruits produced / number of pollinated flowers*) x 100] and assessment the full seed in relation to the total seed [Fertility (%) = (*number of full seeds/total number of seeds (full + empty)*) x 100]. A classification range based on fructification and fertility percentage was used, therefore 0 to 35% was considered low, 36 to 65% intermediate and 66 to 100% high.

Combining ability

The full seeds of the intra- and interspecific crosses of the previous experiments were subjected to mechanical scarification because native *Sphaeralcea* species present physical dormancy in its seeds (Gutierrez *et al*., 2019). Scarified seeds were then germinated in a culture chamber. The seeds that germinated were sown in seedling trays with commercial GROWMIX MultiPro® substrate and cultivated in the greenhouse under controlled light (shading net 50% of light extinction), temperature (18-28°C) and humidity (55-85%) conditions (early growth stage). The seedlings that developed three to four true leaves were transplanted into 7x7x9 cm pots with a substrate composed of 50% sandy soil, 35% peat, 10% perlite and 5% compost (advanced growth stage). Survival of germinated seeds and seedlings from intraspecific offspring (siblings) and reciprocal interspecific offspring (hybrids) were evaluated to quantify combining ability at each stage of development (early growth stage and advanced growth stage). A classification range was used for the percentages of germination and seedling survival as low (0 to 35%), intermediate (36 to 65%) and high (66 to 100%).

Table 2 - Combinations of interspecific reciprocal and intraspecific crosses that originated the hybrid and sibling offspring, respectively

Female parent (♀)	x	Male parent (♂)
Interspecific reciprocal crosses		
S. australis		*S. crispa*
S. australis		*S. mendocina*
S. australis		*S. bonariensis*
S. bonariensis		*S. crispa*
S. bonariensis		*S. australis*
S. bonariensis		*S. mendocina*
S. crispa		*S. australis*
S. crispa		*S. mendocina*
S. crispa		*S. bonariensis*
S. mendocina		*S. crispa*
S. mendocina		*S. australis*
S. mendocina		*S. bonariensis*
Intraspecific crosses		
S. australis		*S. australis*
S. bonariensis		*S. bonariensis*
S. crispa		*S. crispa*
S. mendocina		*S. mendocina*

3. Results

Stigma receptivity

The four species of *Sphaeralcea* had different behaviors in terms of stigma receptivity, reaching different maximum percentages and at different times of the day. *S. bonariensis* showed high values at 8:00 am and sustained over time until 2:00 PM when it was 100%. *S. australis and S. crispa* had similar behaviors with two high peaks of receptivity, at 8:00 am and the maximum at 2:00 PM (99% *S. australis* and 93% *S. crispa*). *S. mendocina* during the morning hours showed a different behavior from the rest, with very low stigma receptivity values with an exponential growth between 12:00 to 2:00 PM where it reached the highest percentage of receptivity (92%). After 2:00 PM, when all the species had their maximum peaks of stigma receptivity, the values began to decrease in different ways. For *S. australis* and *S. bonariensis* the decrease was marked, reaching values of 0% at 6:00 PM. For *S. crispa* and *S. mendocina* it was gradual until 6:00 PM, when receptivity was null (Fig. 2).

Pollen viability

The four species of *Sphaeralcea* obtained high percentages of pollen viability, although these values varied throughout the day and between species. The highest values were recorded at 2:00 PM for *S. australis* (99%), *S. bonariensis* (99%) and *S. crispa* (98%), with no statistically significant differences between

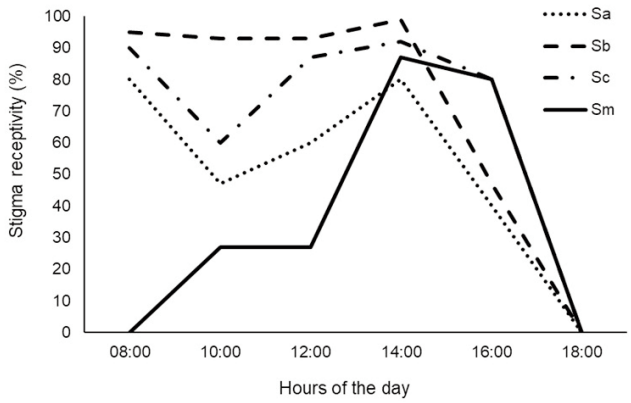


Fig. 2 - Stigma receptivity percentage in *Sphaeralcea australis* (Sa), *S. bonariensis* (Sb), *S. crispa* (Sc) and *S. mendocina* (Sm) flowers in function of time of the day.

them, and at 4:00 PM for *S. mendocina* (99%). At 12:00 PM the percentages were also high for all species and the lowest values were recorded at 6:00 PM for all species (Fig. 3).

Mating system

All combinations, inter and intraspecific crosses, managed to form fruits (Table 3) except *S. mendocina* x *S. bonariensis*. In the case of self-pollinations, fruits were not observed.

The species used as female parent produced differences in the percentage of fruit production. The values were low when *S. crispa* was used as female (7 to 33%), intermediate when it was *S. mendocina* (47 to 53%) and high (67 to 100%) with *S. australis* and *S. bonariensis*. In the intraspecific crosses, the same pattern was repeated, showing low fruiting percentages for *S. crispa* (13%), intermediate in *S. mendocina* (40%) and high in *S. australis* (67%) and *S. bonariensis* (87%).

Regarding fertility, most of the interspecific cross-

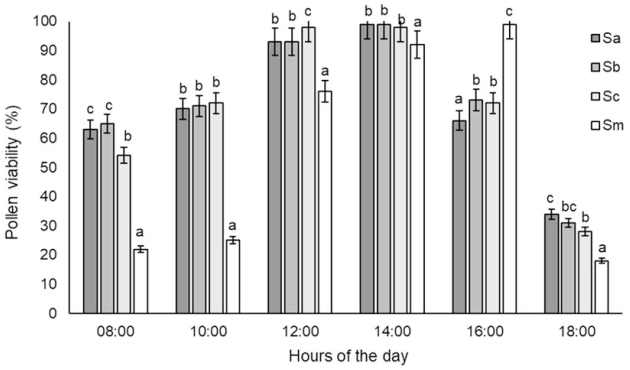


Fig. 3 - Pollen viability percentage in *Sphaeralcea australis* (Sa), *S. bonariensis* (Sb), *S. crispa* (Sc) and *S. mendocina* (Sm) flowers at different times of the day. Means with different letters indicate significant differences with the Fisher's LSD Test, $p<0.05$. Each vertical bar represents mean ± standard error.

Table 3 - Number of pollinated flowers (NPF), number of fruits produced (NFP), fructification percentage (FP), number of full seeds (NFS), number of empty seeds (NES) and seed fertility percentage (SFP) for intraspecific and interspecific reciprocal crosses between *S. australis* (Sa), *S. bonariensis* (Sb), *S. crispa* (Sc), and *S. mendocina* (Sm)

(♀ x ♂)	NPF	NFP	FP (%)	NFS	NES	SFP (%)
			Interspecific reciprocal crosses			
SaxSb	15	15	100	277	43	83.1
SaxSc	15	15	100	188	43	78.3
SaxSm	15	10	66.7	90	68	55.7
SbxSm	15	8	53.3	18	115	4.5
SbxSc	15	15	100	367	43	89.5
SbxSa	15	10	66.7	179	14	90.2
ScxSb	15	2	13.3	14	0	78.6
ScxSm	15	1	6.7	1	9	10
ScxSa	15	5	33.3	68	6	90.5
SmxSc	15	7	46.7	30	8	78.9
SmxSa	15	7	46.7	58	12	79.7
			Intraspecific crosses			
SaxSa	15	10	66.7	121	74	62.1
SbxSxb	15	13	86.7	283	17	94.3
ScxSc	15	2	13.3	21	9	70
SmxSm	15	6	40	63	8	86.7

es yielded high percentages of full seed production with values between 78 to 91%. However, in the cross of *S. australis* x *S. mendocina* it was intermediate (56%) and it was low for *S. bonariensis* x *S. mendocina* (4%) and *S. crispa* x *S*. *mendocina* (10%). For intraspecific crosses, fertility was high for *S. bonariensis* (94%), *S. mendocina* (89%) and *S. crispa* (70%) and intermediate for *S. australis*.

Combining ability

The germination percentage after mechanical scarification was high for all crosses except for *S. australis* x *S. australis*, which showed intermediate values (Table 4). Seedling survival decreased throughout development in all descendants, with high and intermediate values predominating in the first growth stage (plant tray) and the majority being low in the advanced stage of development (pot). In the case of the hybrid, the offspring from *S. australis* x *S. bonariensis* showed the highest values of final survival (56%). In the intraspecific crosses, the highest values of descendant survival were from *S. australis* x *S. australis* (66%). The crosses that failed to develop live seedlings were *S. bonariensis* x *S.*

4. Discussion and Conclusions

The methods used by Osborn *et al.* (1988) and Alexander (1980) to evaluate stigma receptivity and pollen viability, respectively, were effective to achieve successful crosses in the genus *Sphaeralcea*.

Stigma receptivity is a highly variable trait among species of the plant kingdom. There are species such as *Carica papaya* L. where the flowers are receptive before the floral opening and until the closing (Parés *et al.*, 2002), others such as *Passiflora edulis* are receptive during anthesis until flowers closed (Ángel Coca *et al*., 2011). Our results for the genus *Sphaeralcea* showed that *S. australis*, *S. crispa* and *S. bonariensis* had high stigma receptivity at flowers opening, except for *S. mendocina*, which obtained positive results after they opened, and the bubbling was null before flower closure (6:00 PM) for all species. Ambient heat can serve to attract insects to an open flower through volatilization of floral scent during anthesis, and also helps to maintain a period of maximum stigma receptivity (Consiglio and Bourne, 2001). In our results, stigma receptivity was high between 12:00 PM and 2:00 PM which are coinci-

Table 4 - Number of full seeds (NFS), germination percentage (GP), number of seedlings in early growth stage (NSEGS), survival percentage of early growth stage (SPEGS), number of seedlings in advanced growth stage (NSAGS) and survival percentage of advanced growth stage (SPAGS) for intraspecific and interspecific reciprocal crosses between *S. australis* (Sa), *S. bonariensis* (Sb), *S. crispa* (Sc), and *S. mendocina* (Sm)

(♀ x ♂)	NFS	GP (%)	NSEGS	SPEGS (%)	NSAGS	SPAGS (%)
			Interspecific reciprocal crosses			
SaxSb	277	88.8	181	73.6	102	56.4
SaxSc	188	83.5	139	88.5	12	8.6
SaxSm	90	100	90	100	35	38.9
SbxSm	18	100	9	50		0
SbxSc	367	80.5	186	67.4	70	37.6
SbxSa	179	100	77	43	7	9.1
ScxSb	14	100	13	92.9	3	23.1
ScxSm	1	100	0	0	-	
ScxSa	68	69.1	30	63.8		0
SmxSc	30	71.4	7	35	1	14.3
SmxSa	58	92.6	20	40		0
			Intraspecific crosses			
SaxSa	121	63.6	64	85.3	42	65.6
SbxSxb	283	82.3	120	51.5	47	39.2
ScxSc	21	100	11	55	0	0
SmxSm	63	73.8	42	93.3	6	14.3

dent with the time of day when the maximum ambient temperatures were recorded, with an average of 30.4°C (National Meteorological Service, https://www.smn.gob.ar/).

In some species high temperatures affect pollen viability (Rao *et al*., 1992; Radice *et al*., 2020; Iovane *et al.*, 2022). In *Sphaeralcea*, there is still no evidence of how environmental factors affect pollen viability, but our results are indirect evidence that pollen would not be affected by high summer temperatures. One possible explanation for these results is that they are native species adapted to local climate conditions and therefore high temperatures do not generate the thermal stress that affects viability during pollen development or in its mature state. The results of this research indicate that the pollinations that take place between 12:00 PM and 2:00 PM have a greater probability of generating fruits and seeds, since it is when most of the open flowers are receptive, and the viability of pollen is optimal.

These species demonstrated to be self-incompatible and allogamous, with different degrees of reproductive compatibility and combining ability between them. The *S. mendocina* x *S. bonariensis* cross produced aborted fruits and were not able to produce offspring. The crosses *S. bonariensis* x *S. mendocina*, *S. crispa* x *S. mendocina*, *S. mendocina* x *S. australis* and *S. crispa* x *S. crispa* managed to produce viable seeds that germinated, but with no descendants since the seedlings were not fully develop. These effects are probably the product of reproductive incompatibility between the species since they have different chromosome numbers, *S. mendocina* is 2n = 30 and the rest of the *Sphaeralcea* are 2n = 10 (Krapovickas, 1949). It would be interesting to achieve offspring with the germplasm of *S. mendocina* since it has very attractive and particular ornamental features such as the color of the leaves with shades in the range of gray and pink flowers (Gutiérrez *et al*., 2021). The null survival of the intraspecific crosses for *S. crispa* could be due to the rapid loss of vigor of the seeds, since at the time of germination they were smaller plants with a very weak appearance.

Except for *S. bonariensis* x *S. mendocina* and *S. australis* x *S. mendocina* crosses, which were not compatible due to chromosomal differences; the crosses with the best combining ability were those that had *S. australis* and *S. bonariensis* as maternal parent with good fruit production. Regarding combining ability, the crosses with the best survival offspring were *S. australis* x *S. bonariensis* and *S. australis* x *S. australis*. Both produced the greatest quantity and quality of descendant plants that prospered over time and had adequate growth and development. These novel results will allow us to improve the pollination efficiency and to design a strategic plan for the ornamental improvement of *Sphaeralcea genus*

Our study provides the first data on the reproductive biology and mating system of *Sphaeralceae* genus belonging to four native species, which provides valuable information for the formulation and implementation of new approaches for genetic improvement programs. This facilitates the development of new varieties of *Sphaeralcea* hybrids with ornamental qualities.

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Warm stratification combined with organic manure application enhances seed germination and improves *Cycas revoluta* growth and development

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Abstract: *Cycas revoluta* (Sago palm) is one of the widespread ornamental plant, used as an indoor and outdoor plant. Seed propagation is extremely hard and time consuming, given the physical dormancy imposed by hard coat. The use of warm stratification improves seed germination by prompting embryos development. As mean to gain more insight on the beneficial effect of warm treatment on seed germination, histological analysis of warm stratified and untreated embryos was conducted. Our results revealed that warm treatment accelerated embryos development, resulting in a rapid differentiation of embryos' tissues. α-amylase, GA_3 and ABA quantification showed that warm stratified embryos accumulated higher and lower amounts of α-amylase and ABA respectively compared to untreated embryos. Regarding plant development, our results showed that organic manures significantly improved *Cycas revoluta* growth and development. The best response was recorded with the application of sheep manure. Indeed, sheep manure addition increased plant height, the number of leaves per plant, stip length and width by nearby 188% and 61%, 36% and 17% respectively. In roots, the presence of nodules had been recorded in the three applied treatments and more importantly in the presence of sheep manure. At the physiological level, sheep manure supplementation improved photosynthetic apparatus and nitrogen content in leaves (by 75%), thereby explaining the growth promotion. Taken together, these results underlined the beneficial effect of organic manure on *Cycas revoluta* growth and development and proposed a new strategy to improve plant growth and development with the use of sheep manure as organic amendment.

1. Introduction

Cycas revoluta is one of the common ornamental trees, grown in temperate, subtropical and tropical areas of the world, more precisely in Miyazaki and Kagoshima Prefectures in Kyushu District down to the Ryukyu Islands, Okinawa Prefecture in Japan (Dehgan *et al.,* 1994; Zarchini *et al.,* 2011). Taxonomically known as the foremost primitive species among the living cycads, this species has been used as an indoor and outdoor landscape plant over decades (Jones, 1994).

Cycas revoluta can either be propagated from seeds or from vegetative offshoots (Demıray *et al.,* 2017). *Cycas* propagation through seeds is extremely hard due to the physical dormancy imposed by the presence of hard coat (Frett, 1987; Zarchini *et al.,* 2011; Ullah *et al.,* 2020). Moreover, seeds also showed a rapid loss of viability along with a low morphogenic potential, which delays their germination and thus limits their use for rapid and effective propagation (Naderi *et al.,* 2015; Demıray *et al.,* 2017). To overcome these limitations, different pre-germination treatments have been applied to accelerate the germination process where several studies have suggested that germination can be improved by mechanical or chemical scarification (Frett, 1987; Zarchini *et al.,* 2011; Fallahabadi *et al.,* 2012; Ullah *et al.,* 2020). Warm stratification has also been proposed as an efficient strategy to enhance seed germination (Benjelloun *et al.,* 2021).

Besides the problem encountered with seeds' propagation, *Cycas revoluta* is a slow growing species that requires up to 10 years to reach the reproductive maturity (Frett, 1987; Rinaldi, 1999). Nutritional management through organic manures is useful to enhance plant growth, yield and quality (El-Sherbeny *et al.,* 2012; Marak *et al.,* 2020). Organic manures are, by definition, derived from animals, plants and microorganisms. A large panel of manures are nowadays available in the local markets at affordable prices (Khairnar and Kaur, 2022). Organic fertilizers act as slow-release fertilizers, providing nutrients in lower amounts over an extensive time period (Shaji *et al.,* 2021). They are considered as natural source of nutrient supply in the soil and ensure the return of essential macronutrients such as nitrogen (2.42%), phosphorus (1.51%) and potassium (0.41%) as well as micronutrients including calcium, magnesium, manganese and sulphur (Parham *et al.,* 2002; Wang *et al.,* 2010; Khaitov *et al.,* 2019). Organic amendments such as animal, green or composted farmyard manures enhance soil's physical properties by reducing bulk density, improving soil water-holding capacity and increasing infiltration rates (Tester, 1990; Werner, 1997; Gopinath *et al.,* 2008). They also heighten the existing soil nutrients, resulting in the improvement of plant growth by increasing nutrient availability (Shaji *et al.,* 2021).

Organic manures have broadly been used to enhance plant growth in many crop species, including wheat (*Triticum aestivum*), sugarcane (*Saccharum officinarum*), rice (*Oryza sativa*) and maize (*Zea mays*) and in ornamental plants such as marigolds, gladiolus (*Gladiolys grandiflorus*) and roses (Shanmugam and Veeraputhran, 2000; Attiyeh *et al*., 2002; Singh *et al*., 2006; Aziz *et al.,* 2010; Abbas *et al.,* 2012; Soomro *et al.,* 2013; Idan *et al.,* 2014; Baruati *et al.,* 2018). We thought to investigate the effect of organic, sheep and horse manure, on *Cycas revoluta* growth and development by assessing morphological, biochemical and physiological analyses. Besides, as mentioned above, warm stratification enhances *Cycas revoluta* seed germination by accelerating embryos development. However, the beneficial effect of warm temperature on seeds' germination is still not fully understood. Thus, we focused on the histological and the biochemical changes occurring in the warm stratified seeds in comparison with the control, to explain the positive effect of warm stratification on seed's germination.

2. Materials and Methods

Plant material

Freshly harvested seeds collected from 50 years old female mature plants grown in the garden of the Faculty of Sciences, Mohammed V University, Morocco were used in this study.

Effect of warm stratification on embryos development

Warm stratification of *Cycas revoluta* seeds was applied as described by Benjelloun *et al.* (2021). Warm treatment was applied after the mechanical removal of the sacrotesta. Two treatments have been applied: the first treatment (T1) consisted on seed storage at 25°C for 2 months. The second treatment (T2) consisted on seed storage at 30°C for 2 months. Meanwhile, control plot (C) was not subjected to any treatment. The embryos from the control plot are 0 month-old.

Zygotic embryos length and width measurements. Zygotic embryos (ZE) were isolated according the protocol described by Benjelloun *et al.* (2021). ZEs length and width were measured in each condition and the mean was calculated from at least 12 biological replicates.

Zygotic embryos germination. Seeds subjected to the three different conditions (C, T1 and T2) were planted in bins containing sterilized soil at 25 cm depth. Cultures were incubated at 25±2°C, with a photoperiod of 16 hours of light and 8 hours of darkness and watered daily depending on soil moisture. Daily observations were performed and seed emergence was recorded. Percentage of germination was then calculated.

Microscopic observation of zygotic embryos. Microscopic observation of zygotic embryos from C and T2 treatments (2 months-old) was conducted using Epson light microscope equipped with an imaging software. For that, zygotic embryos were fixed using a mixture of 95°C ethanol and acetic acid (3:1) for 24 hours as described by Brhadda and Abousalim (2007). ZEs were dehydrated by passing through a series of alcohol baths (70°, 95° and 100°C). After complete dehydration, ZEs were transferred to two successive bath of toluene for 24 hours. Samples inclusion in paraffin was performed in three successive bath of paraffin maintained at 80°C, each bath lasting 60 minutes. The 10-15 µM thick sections, made with a microtome, were spread on perfectly degreased slides. Sections were stained with 1% toluidine blue and viewed with.

Qualitative assay for alpha-amylase activity. The presence of alpha-amylase activity was assessed according to the method of Xie *et al.* (2007). Zygotic embryos and embryoless half-seeds were placed on 2% agar in 9-cm petri dishes. The agar plates included 0.2% of soluble potato starch, 20 mM CaCl2 and 20 mM Sodium succinate pH 5.0. The petri dishes were then incubated at 28°C for 48 hours. After incubation, I_2/KI solution (2.8 mM I_2+ 43.4 mM KI in 0.2 N HCl) was added to the plates. After 5 minutes, the reaction between starch and iodine turned the agar plates to blue-purple. The agar around ZEs or the half-seeds with alpha-amylase activity remained colourless due to starch hydrolysis triggered by alpha-amylase activity.

Quantitative assay for alpha-amylase activity. Alpha-amylase activity was quantitatively determined according to a slightly modified version of the method of Miller (1959) as described by Liu *et al.* (2018). Isolated zygotic embryos were collected, ground and mixed with 100 ml of chilled distilled water. The mixture was soaked in a cooling bath (4°C) for 10 minutes. The mixture was filtered and the extract was then collected and centrifuged at 12000 rpm for 10 minutes at 4°C. The recovered supernatant was heated for 15 min at 70°C. 1 ml of embryos extract was then mixed with 1 ml of 1% soluble starch dissolved in sodium acetate buffer pH 5.6. The mixture was incubated for 15 minutes at 40°C ad then boiled for 5 minutes in the presence of 2 ml of 3,5-dinitrosalicylic acid. The amount of released reducing sugar was measured using a spectrophotometer at 540 nm using maltose as the reducing sugar standard.

Phytohormones quantification in zygotic embryos. Abscisic acid (ABA) and Gibberillic acid (GA_3) quantification in zygotic embryos was performed on a Finnigan LC-MS/MS system (Thermo Electron, San Jose, CA, USA) consisting of a surveyor autosampler, a surveyor MS pump and a Finnigan LTQ linear ion trap mass spectrometer equipped with an ESI source that was operated in negative mode. The data acquisition software used was Xcalibur. The LC separation was carried out by an HiQ Sil C18 column (250 mm × 4.6 mm i.d., 5 m). The two phytohormones were eluted isocratically with methanol/water containing 0.2% formic acid (50:50, v/v) at the flow-rate of 1.0 mL min^{-1}. The injector volume selected was 25 L. LC-MS/MS conditions were as follows: ESI spray voltage, 4 kV; sheath gas flow-rate, 70 arb; auxiliary gas flow rate, 20 arb; capillary voltage, -38 V; capillary temperature, 350°C and tube lens, 95 V. The SRM mode was used for the determination of the phytohormones. GA_3 and ABA were monitored at m/z transitions of 345→239 and 263→153, 219, respectively. The optimized collision energies for GA3 and ABA were 21 and 20 eV, respectively. Selected ion monitoring (SIM) mode was used for the determination of ISTD. ISTD was monitored at m/z 121.

Effect of soil amendments on plant growth and development

Plant material and soil treatments. Cycas revoluta plants, coming from seeds were grown in greenhouse, in 3L pots. After one year of culture, plants were randomized and divided based on them into three similar groups (corresponding to each one of

the three soil-substrates-treatments). The three treatments were as follows: (i) plants grown soil substrate only, (ii) plants grown on soil substrate, mixed with sheep manure and (soil: sheep manure = 90:10) (iii) plants grown on soil substrate, mixed with horse manure (soil: horse manure=90:10). Sheep and horse manures were produced by Sardi breed and Arabic breed, respectively. The chemical composition of soil and organic manure are represented in Table 1. In each of the three described treatments, 20 plants-replicates (one plant per pot) were included. During the experiment that lasts six months, all the plants were tri-weekly irrigated with distilled water.

Plant growth data recording. At the end of the experiment, number of leaves, plant height, stip length and width, root length and density, the number of nodules per plant, their length and width were measured.

Leaf, stip and root nutrient contents. At the end of the experimental period (6 months), *Cycas revoluta* leaves, stips and roots were washed and dried. They were then ground to a fine powder, to pass a 30-mesh screen. 0.5 g of the fine powder of each sample was dry-ashed at 515°C in a muffle furnace, for 5 hours. The ash was dissolved with 3 ml of 6 N HCl and diluted with double distilled water up to 50 ml. The concentrations of P, Na, K, Fe, Cu and Zn were determined using DTPA method as described by Lindsay and Norvell (1978). Nitrogen content was estimated using the Kjeldahl method. Macronutrient (P, Na, K and N) were expressed in % DW, while micronutrients (Fe, Cu and Zn) amounts were expression in mg/Kg DW.

Chlorophyll fluorescence. Chlorophyll fluorescence measurements were performed using a pulse-modulated fluorometer (OS30p, Opti-Sciences, Hudson, NH, USA). Fluorescence measurements were assessed in dark-adapted leaves, using the leaf-clips which were put on the adaxial leaf blades away from the leaf vein. Two measurements were made on each pot. The following chlorophyll fluorescence parameters were determined: maximal photochemical efficiency of PSII (Fv/Fm), the maximum quantum yield of primary photochemistry (Fv/F0) and quantum photosynthetic yield of PSII.

Chlorophyll a, b and total chlorophyll contents. Chlorophyll content was performed as described by Bassa *et al.* (2012) with a slight modification. 0.25 mg of fresh leaves were randomly taken for each treatment. The fresh tissue was fine grounded in a mortar and pestles in the presence of 80% of acetone. The mixture was then centrifuged in 10000 rpm for 1 minute. Samples were analyzed by spectrophotometry at two wavelengths, 645 and 663 nm, using 80% acetone as the blank. The chlorophyll a, b and total chlorophyll contents were calculated according to the following equations: Chl a=0.999A663-0.0989A645; Chl b= 0.328A663+1.77A645 and total chlorophyll content=20.2*Chl a +8.02*Chl b.

Table 1 - Chemical composition of soil and organic amendments before assays launch

	Soil	Horse manure	Sheep manure
pH	7.64±0.05	8.50±0.01	8.3±0.01
CEC (mS/cm)	0.21±0.02	2.13±0.08	2.15±0.04
Potassium (ppm)	120.02±12.50	612.91±5.56	512.38±2.58
Phosphorus (ppm)	58.45±0.71	330.97±0.99	329.16±0.98
Nitrogen (%)	0.038±0.004	1.87±0.01	2.55±0.01
Organic matter (%)	2.88±0.91	44.8±1.2	46.12±1.07
Dry matter (%)	91.13±0.26	97.08±0.03	98.335±0.06
Magnesium (%)	7.25±0.04	0.65±0.01	0.7±0.01
Carbon (%)	1.81±0.005	105.23±0.01	106.12±0.005
Sodium (m eq/100 g)	1.29±0.06	7.35±0.05	7.65±0.15

Statistical analysis

All the analysed parameters have been compared using a fixed model of analysis of variance (ANOVA). For each parameter and condition, means and standard deviation were calculated based on at least twelve biological replicates (except for α-amylase, ABA and GA_3 amounts for which means were calculated based on three independent biological replicates). In case of significant difference between groups, a Tukey test was used for means separation, at risk of 0.05. The relationship between parameters was observed using Pearson coefficient. A principal component analysis was also launched to determine which parameter contribute most to the variation in data.

3. Results and Discussion

Warm stratification affects alpha-amylase activity, GA_3 and ABA contents and enhances zygotic embryos development and seed germination

Seed germination partially relies on the degradation of storage reserves in mature seeds. Sugars from starch hydrolysis are the major source of energy required for seedling emergence (Beck and Ziegler, 1989). Alpha amylase is the major enzyme involved in

starch mobilization and its degradation into small organic molecules to provide energy and nutrient indispensable for seed germination and seedling emergence (Ali and Elozeiri, 2017). Quantification in treated (T2) and untreated embryos (C) revealed a huge difference in alpha amylase activity between the two treatments. We observed an increase in alpha amylase activity in 30°C warm stratified embryos (T2), as compared to the untreated plot (C) (Fig. 1b). This finding was also confirmed by the qualitative data (Fig. 1a). The colourless areas around embryoless half-seeds derived from untreated (C) seeds were much smaller than those stored at 30°C for 2 months (T2). Previous work showed that alpha amylase activity substantially increased with the increase of temperature. Indeed, Salisbury and Ross (1995) demonstrated that some enzymes like alpha amylase reactions and thus activities increased with temperature increases from 0°C to 35°C. However, above 40°C, enzyme activities decreased due to their denaturation (Salisbury and Ross, 1995). Sari (2021) showed that the highest alpha amylase activities were recorded in rice seeds (*Oryza sativa* var Cisokan) at 30-40°C. They noticed the absence or the decrease in alpha-amylase activity in temperature below 28°C and above 40°C, respectively (Sari, 2021). Here, we reported a notable and significant increase in alpha-amylase activity in warm treated seeds (T2), which can be explained by the beneficial effect of moderate temperatures (30°C in our case) on alpha-amylase enzyme.

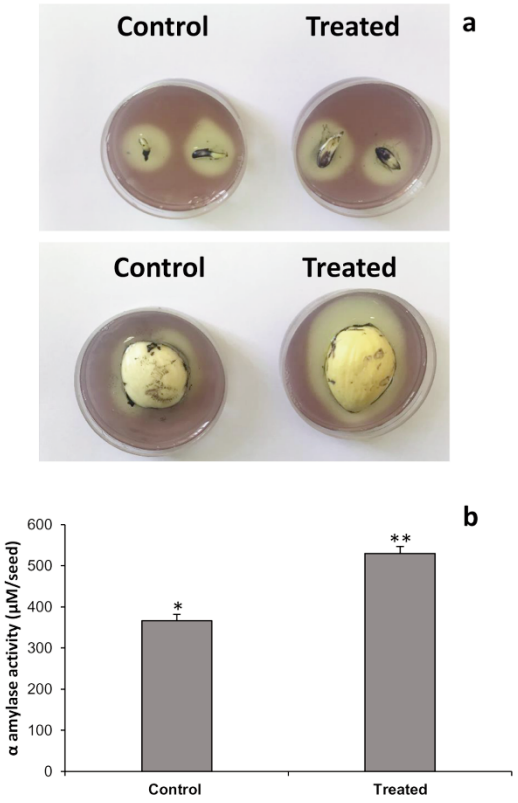

Fig. 1 - Qualitative (a) and quantitative (b) determination of α-amylase activity in untreated (C) embryos and those stored at 30°C for 2 months (T2). The presence of α-amylase was qualitatively determined in embryos and embryoless half-seeds. The quantitative determination of α-amylase activity was performed on untreated and treated embryos. Values are mean ± SD of three biological replicates. Asterisk (*) showed statistical significance according to T-student test ($p<0.05$).

Seed dormancy and germination are mainly regulated by two major antagonist phytohormones, abscisic acid (ABA) and gibberellin (GA). ABA positively regulates the induction and the maintenance of seed dormancy while GA enhances germination (Tuan *et al.,* 2018). Investigating ABA and GA_3 amounts in pre-treated (T2) and untreated (C) embryos of *Cycas revoluta* revealed a notable difference in ABA and GA_3 amounts. Untreated embryos (C) accumulated more ABA than warm stratified embryos (T2) (Table 2). However, GA_3 amount in warm treated (T2) seeds was higher than in the control (C). This difference was although statistically insignificant. It was previously reported that the balance of seed ABA/GA levels is a pivoting regulatory mechanism underlying the maintenance and release of seed dormancy. Seeds 'dormancy studied in *Arabidopsis thaliana* revealed that the suppression of GA_3 biosynthesis and ABA catabolism inhibited seeds ' germination and resulted in dormancy implementation (Chen *et al.,* 2020). The improvement of rice seed germination was linked to the trigger of the glycolytic metabolism and the restoration of GA/ABA balance in seeds (Yang *et al.,* 2022). Warm stratified embryos accumulated more GA_3 than ABA. Besides, as mentioned above, warm treated embryos showed higher alpha-amylase activity, reflecting a strong glycolytic activity. Thus, the lower ABA content along with the increased GA_3 levels and alpha-amylase activity suggest that those embryos can easily germi-

Table 2 - GA_3 and ABA contents in untreated (C) and warm stratified (T2) embryos

Treatments	GA_3	ABA
Untreated embryos (C)	68.91±1.35 (*)	42.09±0.44 (*)
Warm stratified (T2) embryos	65.15±0.81 (*)	30.11±1.29 (**)

Values are mean ±SD of three independent biological replicates. (*) Showed statistical differences according to t-student test ($p<0.05$).

nate and explained the stimulatory effect of warm stratification on embryos germination that has been previously reported by Benjelloun *et al.* (2021).

Cycas revoluta seeds have not the ability to immediately germinate after seed shed. This finding was attributed to embryo immature stage as previously reported in *Cycas rumphii* (De Silva and Tambiah, 1952), *Cycas revoluta* (Dehgan and Schutzman, 1989) and *Eucephalartos natalensis* (Woodenberg *et al.,* 2014). In this study, histological analysis showed that untreated embryos displayed a rudimentary structure subtended by a long suspensor as expected (Fig. 2a). Treated embryos (T2) was although observed to undergo considerable growth and development which confirmed our earlier observation (Fig. 2b) (Benjelloun *et al.,* 2021). Microscopic observation of embryos from control plot (C) or subjected to treatment 2 (T2) thin longitudinal sections stained with toluidine blue revealed that untreated embryos were at early stage of development, which corresponds with early stage of globular embryo (Fig. 2). The

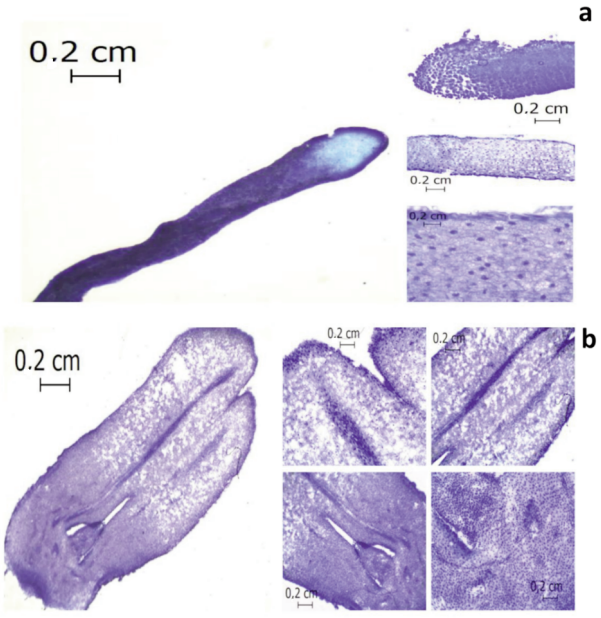

Fig. 2 - Histology of (a) embryos from the control plot (0 month) (C) and (b) embryos stored at 30°C for 2 months (T2). The embryos were stained darkly with toluidine blue. Bar, 200 µm. Observations have been made for the control, using embryos isolated immediately after seed shed. For those subjected to T2 treatment, the observations were performed after 2 months of storage at 30°C. Untreated embryos (C) displayed a rudimentary developmental stage, cells of the embryo are thin, showed small size in comparison with cells of the suspensor (longer cells). Warm stratified embryos (T2) showed a differentiated structure with two apparent cotyledons, shoot and root meristem can be easily identified.

embryo tissue had no intercellular spaces. Cells were bounded by thin walls with prominent nuclei. Warm stratified embryos showed several morphological, histological, and cellular differentiation (Fig. 2). Those embryos were able to reach cotyledonary stage in only 2 months. Shoot and root meristems can be differentiated. Shoot meristem is well developed and flanked by cotyledonary protuberances. Procambium tissue, a meristematic tissue concerned with providing the primary tissues concerned with providing the primary tissues of the vascular system, was well developed. This phenomena has been earlier explained by Devillez in 1976 in *Taxus baccata* by the fact that warm stratification prompted after-ripening in underdeveloped embryos along with the suppression of the morphological dormancy (Devillez, 1976). Chien *et al.* (1998) have found that warm stratification promoted embryos development in *Taxus* species, the embryos reached the double of their size after six months of warm stratification (Chien *et al.,* 1998). This finding is consistent with our previous observation, in which we reported that 2 and 4 months' exposure to warm treatment significantly increases *Cycas revoluta* embryos' length (Benjelloun *et al.,* 2021). Seed germination in dormant seeds generally occurs as a result of the metabolic activation (Bewley and Black, 2013), supplying cells with the energy required for cell differentiation, expansion and development. For instance, Woodenberg *et al.* (2013) showed that *Encephalartos natalensis* developed embryos (at the cotyledonary stage) accumulated high amounts of starch compared to the other stages. They also suggested that the accumulated starch serves more as carbohydrate reserve during germination and seedling establishment than during the embryo growth in the ovule (Woodenberg *et al.,* 2014).

Given the beneficial effect of warm treatment on zygotic embryos germination, we thought to examine growth parameters (length and width) of zygotic embryos deriving from seeds stored at 25°C (T1) or 30°C (T2) for 2 months, in comparison with untreated seeds (C) isolated directly after seed shed (0 month). Our results showed that seed storage for 2 months at 25°C (T1) or 30°C (T2) significantly increased zygotic embryos' length and width, compared to the control. We noticed that ZEs length increased by 126% and 221% with T1 and T2 treatments, respectively (Table 3). Similarly, we were able to record a 5 to 7 times increase in ZEs width, when seeds were stored at 25°C or 30°C respectively. Germination percentage

Table 3 - Effect of warm stratification on zygotic embryos length, width and germination

Parameters	Control (C)	25°C (T1)	30°C (T2)
ZEs length	3.46 ± 1.14 a	7.84 ± 1.29 b	11.14 ± 1.20 c
ZEs width	0.09 ± 0.02 a	0.51 ± 0.04 b	0.68 ± 0.03 c
Germination percentage	25.33 ± 1.63 a	28.00 ± 1.60 a	49.33 ± 1.70 b

Values are mean ± SD of at least 12 biological replicates.
For each analysed parameter, different letters indicate statistical difference according to Tukey test (p<0.05).

was also stimulated with the application of warm treatment. A significant increase in the percentage of germination was reported with the application of T2 treatment. Indeed, the highest germination percentage of 49.33% was recorded with seeds pre-treated at 30°C for 2 months. Meanwhile, no significant differences were detected between the control plot (25.33%) or T1 treatment (28.00%). These data strongly showed the stimulatory effect of warm treatment on ZEs development and germination, thereby confirming our earlier observations (Benjelloun *et al.,* 2021).

Organic amendments stimulate Cycas revoluta growth

Investigating the effect of soil organic amendments on *Cycas revoluta* growth in greenhouse conditions revealed a stimulatory effect of organic manures on plant growth (Fig. 3).

All fertilization treatments increased plant growth attributes except for root length. We found that both horse and sheep manures significantly increased plant height and stip length, but no significant differences were recorded between the different manures (Table 4).

Fig. 3 - Effect of organic amendments on *Cycas revoluta* growth. (Control) one-year-old *Cycas* plant grown in soil substrate only, (HM) one-year-old *Cycas* plant grown in a mixture of soil and horse manure and (SM) one-year-old *Cycas* plant grown in the presence of mixture of soil and sheep manure. Scale bar = 13 cm.

Regarding root development, a significant increase in root length and root density was observed with the use of sheep manure. Nodules' number also increased in a significant way with sheep manure amendment, while comparable values were recorded in the control treatment and in the presence of horse manure (Table 4). Besides nodules 'number, nodules' length and width was notably variable. Significant differences in nodules' number have been recorded between the three culture conditions.

Table 4 - Effect of organic amendments (horse and sheep manures) on the agro-morphological parameters of *Cycas revoluta* plants

Parameters	Soil	Soil-horse manure	Soil- sheep manure
Number of leaves per plant	1.69 ± 0.48 a	2.94 ± 0.99 b	2.73 ± 0.47 b
Plant height (cm)	20.17 ± 2.40 a	57.43 ± 4.53 b	58.14 ± 2.91 b
Stip length (cm)	3.19 ± 0.32 a	4.35 ± 0.34 b	4.34 ± 0.42 b
Stip width (cm)	3.52 ± 0.34 a	4.15 ±0.38 b	4.17 ± 0.32 b
Root length (cm)	21.14 ± 1.68 a	21.11 ± 3.60 a	32.02 ± 2.51 b
Root density	1.13 ± 0.23 a	1.23 ± 0.20 ab	1.49 ± 0.29 b
Number of nodules per plant	3.18 ± 1.93 a	4.31 ± 1.19 a	12.67 ± 2.05 b
Nodules length (cm)	0.81 ± 0.12 a	0.95 ± 0.19 b	1.22 ± 0.21 c
Nodules width (cm)	0.84 ± 0.12 a	1.39 ± 0.30 b	1.35 ± 0.35 b

Values are mean ± SD of at least twelve independent replicates.
For each parameter, values with different letters indicate statistical differences according to Tukey test (p<0.05).

The highest nodules' length values were recorded in the presence of sheep manure. Regarding nodules' width, the statistical analysis revealed a significant difference between the control and the different manures treatments. The highest width values were interestingly reported with the use of horse manure. The cross-section of these nodules revealed the presence of blue-green halo, which can potentially indicate the presence of cyanobacteria (Fig. 4). Further analysis should be conducted to confirm these observations.

Organic amendments have the ability of binding minerals like, magnesium, potassium and calcium in a colloidal form (humus and clay), which can promote the formation of stable aggregate of soil particles at desired porosity to support plant growth (Azarmi *et al.,* 2009; Chang *et al.,* 2010). Here, we found that organic manures (sheep or horse manures) significantly promoted plant height, number of leaves per plant and stip length. Indeed, manure supplementation increases macro and micronutrient contents as well as soil physico-chemical properties, which can ultimately lead to a better vegetative growth (Adekiya *et al.,* 2020). Root development can be significantly influenced by soil mineral composition. The presence of sufficient nutrients prompted the development of root system. Gregory (1994) compared the influence of fertilization on root growth to its beneficial effect on shoot growth. Smith showed that additional nitrogen levels can result in a better leaf growth and number of another cycadales species; *Zamia integrifolia* (Smith, 1978). Here, we found that sheep manure allowed a better development of root system, as evaluated by root length and density (Gregory*,* 1994). In quinoa (*Chenopodium quinoa*), Kakabouki *et al.* (2019) have linked the better development of root system in plants grown in a fertilized soil with the presence of high amounts of nitrogen (Kakabouki *et al.,* 2019). Sheep manure used in this study displayed the highest nitrogen content (2.55 ± 0.01%) (Table 1), thus explaining the better plant development recorded. Besides promoting shoot and root development, sheep manure supplementation resulted in a significant increase in the number of nodules per plant (Table 4). It has been previously reported that *Cycas revoluta* forms beneficial association with the blue green algae, ensuring nitrogen fixation. This symbiotic nitrogen fixation, occurring in cycads coralloid roots has been reported to arise nitrogen at a significant rate (Halliday and Pate, 1976; Grove *et al.,* 1980). Nitrogen fixation by coralloid roots was estimated to be comprised between 18.8 kg N/ha and 35 kg N/ ha (Smith, 1978). Dehgan (1983) showed that nitrogen fixation in the coralloid roots contributes significantly to plant growth (Dehgan, 1983).

Fig. 4 - *Cycas revoluta* plant presenting a globular-like structures or nodules in the underground part of the plant. (a) *Cycas revoluta* root system with several nodular structure, (b) a single nodule and (c) isolated nodule presenting a blue-green halo which indicates the presence of cyanobacteria. black narrow pointing the blue-green halo. Scale bar = 1 cm.

The presence of great number of nodules per plant in *Cycas* plants grown in the presence of sheep manure could indicate a better nitrogen fixation potential, which can increase plant supplying with sufficient amount of nitrogen. Besides cyanobacteria - *Cycas* roots association, previous reports showed also the presence of endophytes in regular and coralloid roots of *Cycas bifida* (Zheng *et al.,* 2018). Endophytic bacteria have been associated with the growth promotion of several crop species such as maize (Alkahtani *et al.,* 2020), wheat (Khan *et al.,* 2017), tomato (*Solanum lycopersicum*) (Amaresan *et al.,* 2012), rice (*Oryza sativa*) (Khan *et al.,* 2020) and chilli (*Capsicum annuum*) (Amaresan *et al.,* 2012). The plant growth is promoted through improved nutrient acquisition, including nitrogen fixation and the production of plant growth promoting substances such as indole acetic acid and cytokinins (Miliute *et al.,* 2015). Therefore, it could be more interesting to investigate the presence of these beneficial microorganisms in the observed nodular structures, reported in this work.

Organic manures have a positive effect on Cycas revoluta seedlings photosynthetic apparatus

Chlorophyll a, b and total chlorophyll contents significantly increased with the application of different organic manures (Fig. 5). An average increase (2.11 and 2.67 mg/g FW) of chlorophyll a content was recorded with the application of horse manure and sheep manure respectively. Similar trend was also observed with chlorophyll b (3.61 and 4.61 mg/g FW) and total chlorophyll (71.06 and 82.98 mg/g FW) contents. Regarding the chlorophyll fluorescence, our results showed an increase in chlorophyll fluorescence attributes mainly the potential activity of PSII, the maximum quantum yield of primary photochemistry and effective quantum yield of PSII with the application of either horse or sheep manure (Fig. 5). Note that no significant difference has been observed in the potential activity of PSII between the control and plants grown in horse manure-soil mixture.

It is well established that organic fertilizers improved plant growth and development, by improving soil physico-chemical properties and nutrient availability to plants (Eneji *et al.,* 2001; Azarmi *et al.,* 2009; Osama *et al.,* 2016). This latter seems to directly affected photosynthesis process (Osama *et al.,* 2016). Several studies conducted on different plant species such as sugarcane (*Saccharum officinarum*), Soybean (*Glycine max*), Potato (*Solanum tuberosum*) and Kiwifruit (*Actinidia deliciosa*) have underlined the positive effect of organic amendments on chlorophyll

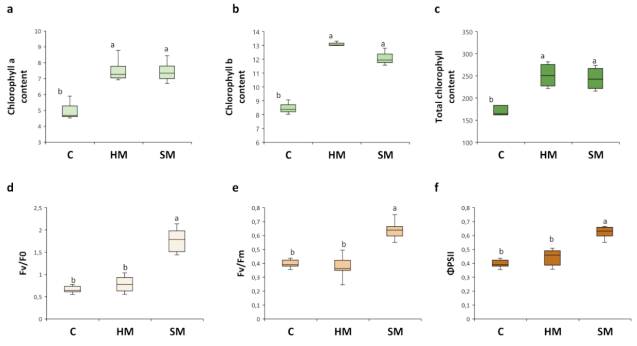

Fig. 5 - Effect of organic amendments - horse manure (HM) and sheep manure (SM) - on the photosynthetic attributes. (a) chlorophyll a content, (b) chlorophyll b content, (c) total chlorophyll content, (d) the maximum quantum yield of primary photochemistry (Fv/Fm), (e) potential activity of PSII (Fv/Fo) and (f) effective quantum yield of PSII (ΦPSII) of *Cycas revoluta* plants. Values are mean ± SD of at least six independent replicates. For each parameter, values with different letters indicate statistical differences according to Tukey test ($p<0.05$).

content (Ghosh *et al.,* 2004; Bokhtiar and Sakurai, 2005; Najm *et al.,* 2012; Sharma *et al.,* 2022). Sharma *et al.* (2022) associated this beneficial effects on photosynthetic properties with the presence of high nitrogen amounts conferred by organic manure supplementation (Sharma *et al.,* 2022). Moriwaki *et al.* (2019) explained the positive effect of nitrogen on photosynthetic attributes (photosynthetic quantum yield) by the increase in thylakoid density, which can enhance green light absorption (Moriwaki *et al.,* 2019). In *Cycas revoluta*, we found that organic manures, more precisely sheep manure, significantly improve plant photosynthetic attributes (Fv/Fm and ΦPSII). This can likely be attributed to the high nitrogen content of sheep manure along with the presence of great number of nodules per plant.

Organic manures modify mineral allocation in Cycas revoluta plants

The mineral contents of *Cycas revoluta* botanical parts (leaf and root) were determined after six months of culture. Our results showed a huge difference in macronutrients and micronutrients amounts between the control plants and those grown in the presence of either horse or sheep manure (Table 5). Regarding sodium content, the highest and lowest sodium amounts was recorded in roots grown in the presence of sheep manure and leaves collected from the control plants. Potassium content was highly variable between the three different treatments. The highest potassium content of 15.02±2.18% was detected in roots isolated from plants grown in the presence of sheep manure. Meanwhile, the lowest potassium content of 3.2±0.64% was this time recorded in leaves under sheep manure treatment. The highest nitrogen content was recorded in roots of the control plants and leaves collected from plants grown in the presence of sheep manure. However, no significant differences have been recorded in phosphorus amount. For iron, the highest and the lowest amounts of 7.02±0.39 mg/Kg and 11.83±0.50 mg/Kg were observed in leaves collected from plants grown in the presence of sheep manure and roots of the control plants, respectively. The highest copper and zinc contents were recorded in leaf under horse manure treatment and root of the control plants respectively while the lowest amounts were found in roots of the control plots and roots under horse manure treatment, respectively.

Organic fertilization is known to affect the concentrations and the uptake of several macro and

Table 5 - Mineral composition of roots and leafs of *Cycas revoluta* plants subjected to the three following treatments (Control "C", Horse manure "HM" and Sheep manure "SM") for six months

Treatments	Nitrogen (%)	Phosphorus (%)	Sodium (%)	Potassium (%)	Iron (mg/Kg)	Cupper (mg/Kg)	Zinc (mg/Kg)
Root							
C	1.75±0.09 a	0.33±0.05 a	1.50±0.36 a	4.55±0.15 cd	11.56±0.21 a	46.33±2.08 d	338.66±9.08 a
HM	1.44±0.09 c	0.36±0.08 a	1.95±0.26 a	11.42±1.11 b	10.99±0.38 ab	49.83±2.83 d	89.90±3.34 d
SM	1.60±0.14 ab	0.43±0.08 a	4.03±0.77 b	15.02±2.18 a	11.15±0.52 ab	47.50±3.44 d	168.0±15.93 c
Leaf							
C	1.19 ±0.07 d	0.43 ±0.05 a	1.12 ±0.13 a	4.55 ±0.05 cd	7.92 ±0.72 c)	67.0 ±2.00 bc	282.50 ±4.5 b
HM	1.01 ±0.11 de	0.38 ±0.09 a	1.40 ±0.20 a	10.50 ± 1.21 b	8.12 ±0.59 c	85.00 ±3.60 a	166.33 ±18.55 c
SM	1.77 ±0.18 a	0.35 ±0.05 a	2.31 ±0.10 a	2.68 ±0.08 d	7.02 ±0.37 d	61.00 ±1.82 c	245.50 ±24.49 b

Values are mean ±SD of three independent biological replicates. Values with different letters indicate statistical differences according to Tukey test ($p<0.05$).

micronutrients such as nitrogen, potassium and phosphorus by plants, independently from the irrigation system (Yang *et al.,* 2004). The highest nitrogen amounts were recorded in the aerial parts of *Cycas* plants grown in the presence of sheep manure, thus explaining the positive effect of sheep manure supplementation on vegetative growth and photosynthetic attributes. Potassium concentration was significantly more in the roots isolated from plants grown in the presence of either sheep or horse manure than in plants grown without organic manure addition. Maximum increase of leaf K amount was detected with the addition of horse manure. The increase in K concentration, resulting from organic matter addition might be attributed to K concentration in the organic fertilizers (Table 1), as previously shown for *Zea mays* (Aziz *et al.,* 2010) and *Brassica juncea* (Aziz *et al.,* 2006).

The interrelationship between plant morphological, biochemical, and physiological attribute using the Pearson's correlation matrix revealed the existence of a significant positive correlation of potassium content in roots with leaves number (r=0.89), stip width (0.75), nodules' number (r=0.72), nodules' length (r=0.59), nodules' width (r=0.54), chlorophyll a (r=0.77), chlorophyll b (r=0.81) and total chlorophyll (r=0.78) contents. Similarly, root sodium content was positively correlated with root length (r=0.81), nodules' number (r=0.80), their length (r=0.61) and root potassium amount (r=0.68). Root sodium amounts were also positively correlated with photosynthetic attributes, more precisely with Fv/F0 (r=0.84), Fv/Fm (r=0.76) and PSII (r=0.79) (Fig. 6a). Interestingly, root zinc amounts were negatively correlated with stip length (r=-0.78) and width (r=-0.59), leaf length (r=-0.9), chlorophyll a (r=-0.84), chlorophyll b (r=-0.96) and total chlorophyll contents (r=-0.87). PCA (Principal component analysis) showed that variables explained 66.9% of the variation in the first two axes (Fig. 6b), which is why 42.6% and 24.3% variances were accounted respectively, for the first and second principal components. The first principal component counted more attributes than the second principal component. Results from this study strongly suggest that the application of sheep manure significantly affected the plant root growth (root length), the photosynthetic attributes (Fv/Fo, PSII, Fv/Fm) and also root sodium content. Horse manure seems to strongly affected copper amounts in both leaves and roots.

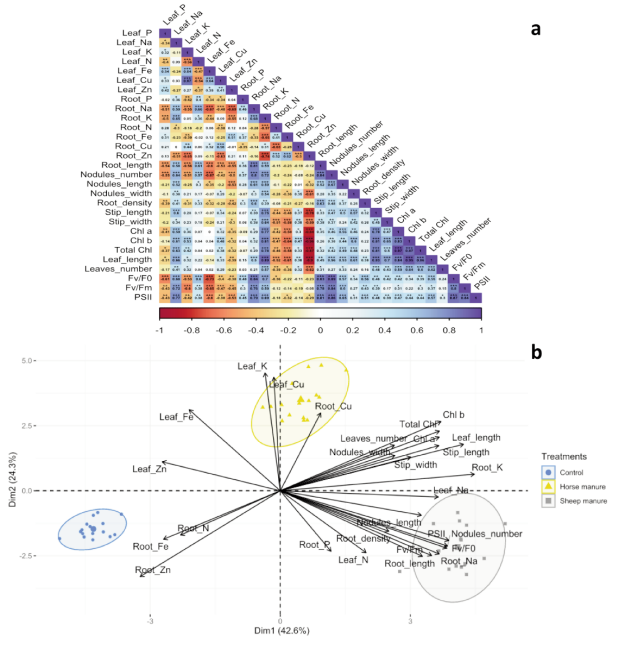

Fig. 6 - Pearson correlation (a) and Principal component analysis (b) gathering all the agro-morphological and physiological parameters that have been used for this study.

4. Conclusions

Here, we demonstrated that warm stratification significantly enhanced *Cycas revoluta* seeds' germination by promoting embryos' length. In the present work, we found that warm stratification increased α-amylase (key enzyme involved in starch mobilization and degradation) activity triggered by the moderate temperature imposed during the warm treatment application. Decreased ABA content was also detected in warm stratified embryos, thus explaining the highest germination percentage recorded in the previous work (Benjelloun *et al.,* 2021). At the histological level, we found that warm stratification triggers cell differentiation and embryos development to reach cotyledonary stage after 2 months of treatment only. Plant growth was highly stimulated by the application of organic manures, more specifically by sheep manure application. Indeed, sheep manure application improves plant growth, namely plant height, root length and density, the number of nodules per plant, nodules 'length and width, nitrogen allocation and photosynthetic attributes. Overall, these data strongly recommend the use of warm stratification to enhance *Cycas revoluta* seed's germination followed by sheep manure application to accelerate *Cycas revoluta* growth, thereby offering new insights on the use of biological agriculture inputs for sustainable production of horticultural plants.

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Yield related traits in some Persian walnut cultivars: Analysis of genetic and genetic by environment interaction

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Abstract: The most important trait in tree species, including walnut, is the yield. In this study, the effect of genotype and their interaction with year on Nut weight, Kernel weight, Kernel percentage, Fruit set, Nuts number on Scaffold (Canopy) Cross Area (SCA), Nut weight on SCA and Kernel weight on SCA were evaluated on Caspian, Persia, Alvand, and Chaldoran walnut cultivars. The results showed that the effects of year, genotype, and year × genotype interaction on all traits were significant. The results showed that Alvand had the highest number of nuts (41.8 per m^2) and nut weight (472.1 g/m^2) on (SCA). Heritability (H^2_b) for kernel weight and kernel percentage, were estimated 0.75 and 0.80, respectively. The lowest value of H^2_b (0.36) was belong to fruit set. The analyses of genetic and phenotypic correlations between traits showed that, the nut weight had (r_g = 0.31, r_p = 0.27) a moderate correlation with SCA same as kernel weight (r_g = 0.34, r_p = 0.29). The GGE biplot analysis explained most of the existing variations (>90%). The genetic effect (PC_1) for all traits were higher respect to the genetic × environment interaction (PC_2), especially for the kernel percentage (94.4%) and number and weight of nut and kernel on SCA (>90%). The lowest value of the PC_1 was related to the fruit set (65.6%), which indicates the trait was more affected by genetic × environment interactions (21.8%). So, this result showed that the yield-related traits in walnut is highly relevant to environment(year in this study) and evaluation of the new cultivars needs careful attention in this case.

1. Introduction

The accurate identification of genotypes is a basic requirement for appropriate utilization of germplasm in practical breeding programs. The diverse climatic conditions, environment, and their interactions with

genetic are the most important factor determining the performance of the cultivars (Fehr, 1987). Therefore, the genetic and environment implies the differential performance of genotypes that rises from the variations in the genotype's sensitivities to the environmental conditions (Rawandoozi *et al.*, 2021). Change of climate not only affect the phenology of tree species, but also affects its production. So, with considering the climate changes and abiotic stresses, walnut production in the world has encountered challenges more than ever before. On the other hand, selection for fruit quality traits is complex; because the most of these traits are often controlled by several loci that are also influenced by the environment (Bliss, 2009). Nut and kernel weight as well as fruit set percentage could be considered as walnut yield components, while the yield efficiency could include nut and kernel weights produced on trunk cross area (TCA) or scaffold cross area (SCA) (Mahmoodi *et al.*, 2015; Dogra *et al.*, 2018; Hassani *et al.*, 2020 b). These traits can be affected by environmental conditions in several ways. For example, the climatic factors affect the receptivity period of walnut pistillate flowers and therefore affect the fruit set percentage and yield of walnut trees (Mariana and Sina Niculina, 2017).

Dogra *et al*. (2018) calculated phenotypic and genetic broad sense heritability of walnut yield related traits. Based on their study the pistillate flower density, fruit set percentage, circumference and cross section of tree trunk showed the highest correlation with the yield. Some walnut trees somewhat show different alternate bearing habits, so the yield is affected by the crop load of the previous year (Mahmoodi *et al*., 2015). Marrano *et al.* (2019) reported that lateral bearing habit have a significant influence on yield of walnuts. Besides the leafing date had high heritability (88%) and was therefore recommended as a reliable character for improvement of new cultivars.

Combining analysis of variance and stability analysis could determine the contribution of genetic, environment and their interactions in traits. In spite of climate change is becoming a bigger challenge every day, determining the genetic and environmental effects can led to understand the response of the cultivars to different environments and select the appropriate cultivars for specific environments and eventually to deal better with changing climate (Bliss, 2009; Rawandoozi *et al*., 2021).

Research with number of genotypes evaluated in different locations and years, makes the genetic × environment analysis a major contest. The GGE biplot analysis is a beneficial tool for data analyzing in multi environment trials (Yan and Tinker, 2006). Rawandoozi *et al*. (2021) estimated the variance components, genetic × environment interaction and heritability of fruit quality related traits in nine peach and nectarine low to medium chill F_1 full-sib families together with their parents in two locations. Based on their research the ripe date and fruit development period had high narrow sense heritability. Fruit weight and shape showed the lower heritability.

Scariotto *et al.* (2013) based on budburst percentage and fruit-bearing shoot formation, evaluated the compatibility and stability of peach genotypes in four years. Arji (2018) investigated the stability of yield components of olive cultivars for three years.

Despite the high priority for data availability regarding the climatic adaptability of walnut cultivars, there is need for a continuous basis research with the newly released cultivars. Therefore, this study is conducted to evaluate the adaptability of some new Persian walnut cultivars to determine the variance components and cultivars adaptability affecting the yield components and yield efficiency traits, especially with increasing the climate change challenge.

2. Materials and Methods

Plant materials and location

Walnut yield component together with the yield efficiency traits in four newly released cultivars (i.e., Caspian, Persia, Alvand, and Chaldoran) (Hassani *et al*., 2020 b) with Chandler and Jamal as reference cultivars, were evaluated in three consecutive years (2015-2017). The cultivars, grafted on Persian walnut seedlings rootstocks, were planted in Karaj in 2006 (35.76031 N, 50.96833 E; elevation: 1240 m a.s.l.; mean annual temperature: 15.8°C; and mean annual precipitation; 247 mm).

Evaluated traits

The data were recorded on yield component traits including nut and kernel weight and fruit set percentage together with the yield efficiency traits such as: nut and kernel weights produced on scaffold cross area (SCA). To estimate the number of pistillate flowers and fruits on experimental trees, pistillate flowers and fruits were counted in sample branches and then were used to predict the whole trees using regression.

To measure Scaffold Cross Area (SCA), the tree's canopy diameter was measured. The SCA was then

estimated using the canopy and the circle approximation. For nut and kernel traits, 30 samples in each treatment were evaluated. The tree nut and kernels' yield were obtained from the number of nuts per tree multiplied per average nut and kernel weights. Next, the nut and kernel yield of trees were divided by the corresponding SCA's, for estimating yield efficiencies based on nut and kernel (Hassani *et al.*, 2014). To calculate the fruit set percentage, the fruit number in sample branches were divided by the corresponding number of pistillate flowers.

Statistical analysis

The combined analysis of variances was carried out using general linear model (GLM) procedure. Means were separated by Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD). Phenotypic (σ^2_p), Genetic ($\sigma^2 g$) and genetic × year interaction (σ^2_{gy}) variances were obtained from their corresponding expected mean square in ANOVA table. Heritability in the broad sense (H^2_b) was estimated using the genetic and phenotypic variances (Visscher *et al.*, 2008). The phenotypic and genetic correlations were estimated using the variances and variance-covariance matrices of traits (Dogra *et al*., 2018; Marrano *et al*., 2019). The GGE biplot analysis was employed to determine the year and genotype interaction, besides the combining analysis of variances (Yan and Tinker, 2006).

3. Results

Yield-related traits variability and analysis

The descriptive statistics of the traits were reported in Table 1. The nut weight varied from 8-15.3 g with the average of 11.2 g, while the kernel weight average was 5.8 g varying from 3.9-8.3 g. Though the variation in kernel percentage range were 39.4-66.7%. The fruit set average was 48.7%, with a wide range variation (14-82%) in different cultivars. Mean number of nuts on SCA were 26.8 with a range of 2.7-64.7. Moreover, the average of nut and kernel weight on SCA were 295.1 and 157.4 g/m^2, respectively. Nut weight on SCA ranged 28.8-782.9 g/m^2, while the kernel weight on SCA ranged 12.1-409.7 g/m^2. In general, a high variation was observed for the evaluated traits in different cultivars.

The three years combined analysis of variance and genetic variance components for the studied traits are shown in Table 2. The effect of the year was significant on fruit set percent, the nut number on SCA

Table 1 - Descriptive statistics of the traits evaluated in walnut cultivars

Evaluated traits	Min	Max	Range	Mean	Variance
Nut weight (g)	8	15.3	7.3	11.2	2.9
Kernel weight (g)	3.9	8.3	4.4	5.8	1.3
Kernel percentage	39.4	66.7	27.3	52.1	54.5
Fruit set percentage (%)	14	82	68	48.7	264.1
Nut number on scaffold cross area (no./m^2)	2.7	67.4	64.7	26.8	271.6
Nut weight on scaffold cross area (g/m^2)	28.8	782.9	754.1	295.1	29655.1
Kernel weight on SCA (g/m^2)	12.1	409.7	397.6	157.4	9448.5

Table 2 - Combined analysis of variance, genetic variance components and broad-sense heritability (H^2_b) of the traits in six walnut cultivars (2015-2017)

Variance component	DF	Nut weight mean squares	Kernel weight	Kernel percentage	Fruit set percentage	Nuts number on SCA	Nut weight on SCA	Kernel weight on SCA
Year	2	10.3 NS	0.99 NS	16.8 NS	995.4 *	1149.8 *	159004 *	47838 *
Replication (year)	6	1.3	0.18	17.1	142.8	70.1	5671.1	2319.3
Genotype	5	16.2 **	10.9 **	430.9 **	1197.8 *	1056.4 *	141510 *	46238 *
Year x genotype	10	3.17 **	0.71 **	21.8 **	330.3 **	239.9 **	30441 **	10044 **
Error	30	0.47	0.19	6.1	95.3	76.7	7912.8	2300.1
Cv (%)		6.1	7.5	4.7	20.1	22.9	30.1	30.5
H^2_b	-	0.41	0.75	0.80	0.36	0.42	0.44	0.45
SE	-	0.1	0.025	0.015	0.13	0.11	0.104	0.11

**, * and NS show statistical significance at the probability level of 1%, 5% and not significant, respectively.
SCA = Scaffold cross area; Cv = coefficient of variance; H^2_b = broad-sense heritability and SE = Standard error of H^2_b.

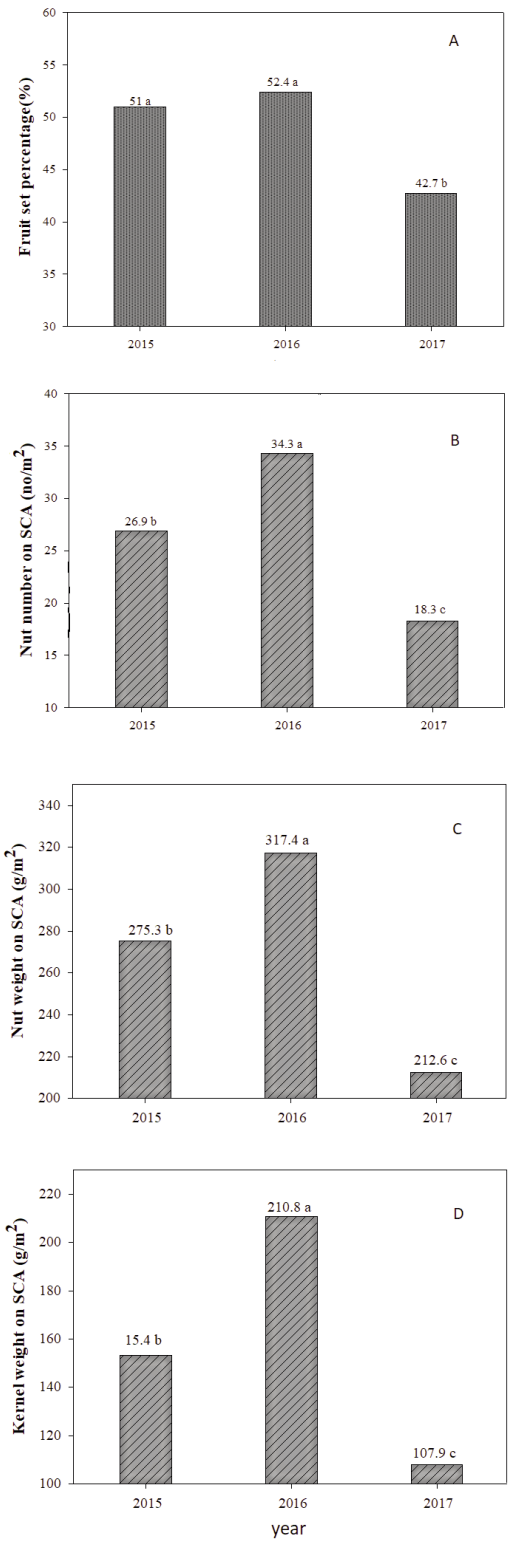

Fig. 1 - Mean comparisons for the effect of years on fruit set percentage (a), nut number on scaffold cross area (SCA) (b), nut weight on SCA (c), and kernel weight on SCA (d).

and the nut and kernel weight on SCA ($P \leq 0.05$). However, it was not significant on nut weight, kernel weight and kernel percent. The effects of genotype and year × genotype interaction was statistically significant in all traits (Table 2).

A high broad-sense heritability (H^2_b) obtained for kernel weight (0.75) and kernel percentage (0.80). The lowest value of H^2_b (0.36) was belong to fruit set (Table 2).

Based on the analysis of results for determining the effect of different years the highest fruit set percentage was observed in 2016 and 2015 with the average of 52.4% and 51%, respectively, although, the lowest fruit set percentage was 42.7% in 2017 (Fig. 1). The highest number of nuts on SCA; and nut and kernel weight on SCA were attained in 2016 with the corresponding averages of 34.3 nuts per m^2; and 317.4 and 210.8 g per m^2.

Evaluation of traits in different genotypes during three experimental years showed that the average of nut weight varied from 9.2 g in Caspian to 13.2 g in Chaldoran. Kernel percentage varied from 42.2% in Chandler to 60.1% in Persia. Fruit set ranged from 33.8% in Persia to 62.7% in Jamal. Furthermore, fruit set was significantly lower in late leafing cultivars and genotypes such as Persia, Chandler, and Caspian (Marrano *et al.*, 2019, Hassani *et al.*, 2020 a) compared with early to medium leafing ones (33.8-43.5% and 55.6-62.7%, respectively) (Fig. 2).

Based on the results a wide range of differences was observed in yield efficiency traits (number of nuts per m^2 SCA and weight of nut and kernel per m^2 SCA). The highest number of nuts per m^2 SCA was observed in Alvand with an average of 41.8 nuts/m^2, while the lowest amount was recorded in Jamal with 9.1 nuts/m^2. Moreover, the highest nut weight on SCA were observed in Alvand with 472.1 g/m^2. Alvand and Chaldoran had the highest kernel weight on SCA with 239.7 and 218.6 g/m^2, correspondingly. The lowest nut and kernel weight on SCA with 108.2 g/m^2 and 50.4 g/m^2 belonged to Jamal (Fig. 2).

Genetic and genetic per environment (GGE) analysis

According to statistically significant interactions between years and genotypes, the studied cultivars showed different responses to years. Analyzing the effect of genotypes and genotype × year interaction on the studied traits have been shown in GGE biplot diagrams in figure 3. In GGE biplot diagrams, the horizontal axis (PC_1) shows the effects of genotypes and the vertical axis (PC_2) shows the interaction of genotype per year (environment). According to the results for nut weight (Fig. 3 a), the PC_1 and PC_2 explained respectively 79.5% and 19.2% of variability, with the

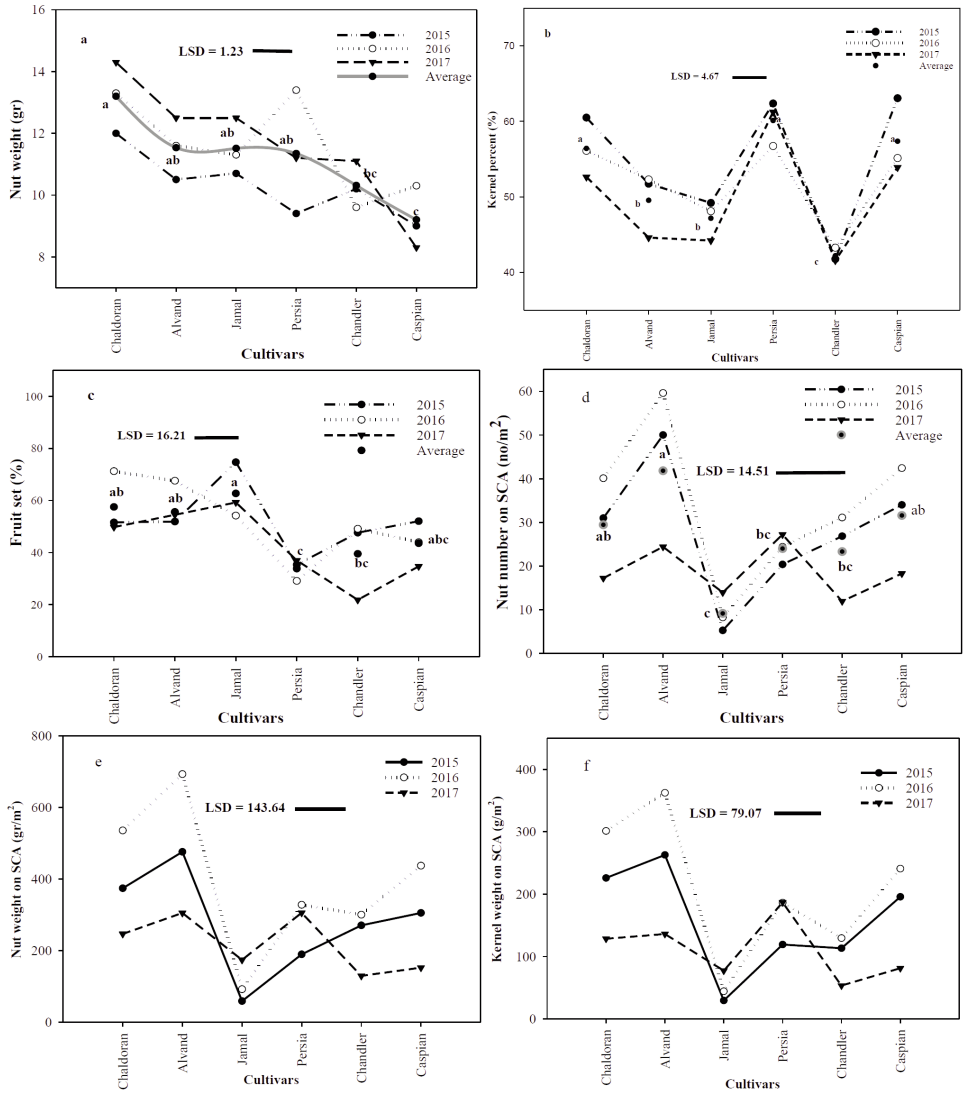

Fig. 2 - Mean comparisons of the effect of the cultivar and year × cultivar on fruit weight (a), kernel percent (b), fruit set (c), number of fruits on SCA (d), fruit weight on scaffold cross area (SCA) (e), and kernel weight on SCA (f) using Duncan multiple range test for genotypes and Least Significant Difference (LSD) test for interaction of year × cultivar.

98.7% of total variability. The biplot 2a was divided into four sectors with a principal sector grouping the years 2015 and 2017, together with Chaldoran and Alvand with higher nut weight. While in the second sector, the year 2016 was grouped with Persia. For the percentage of kernel (Fig. 3 b), the PC_1 and PC_2 explained respectively 94.4% and 4.7% of variability, with 99.1% of total variability in five sectors. The principal sector grouped the years 2015 and 2017. Persia with high kernel percentage was included in this sector. The second sector were grouped the year 2016 together with Chaldoran and Caspian. These cultivars had greater kernel percentage than general average. In figure 3 c the PC_1 and PC_2 explained respectively 65.6% and 21.8% of variability, with 87.4% of total variability about fruit set percentage. The biplot for fruit set percent was divided into five sectors, too. The principal sector grouped the years 2015 and 2017, and Jamal with higher fruit set. The second sector grouped the year 2016 and Chaldoran. This cultivar had fruit set greater than average. For the fruit number on SCA (Fig. 3 d), the PC_1 and PC_2 explained 94.2% and 5.5% (99.7% of total) of variability correspondingly. The GGE biplot was divided into four sectors. In the principal sector the years 2015 and 2016, and the cultivars Alvand, Caspian and Chaldoran were classified together with higher fruit number on SCA. In the second sector, the year 2017 and Persia were grouped together. Similar results were obtained for nut weight on SCA (Fig. 3 e). For the kernel weight on SCA (Fig. 3 f), the PC_1 and PC_2 explained respectively 90% and 9.4% of variability

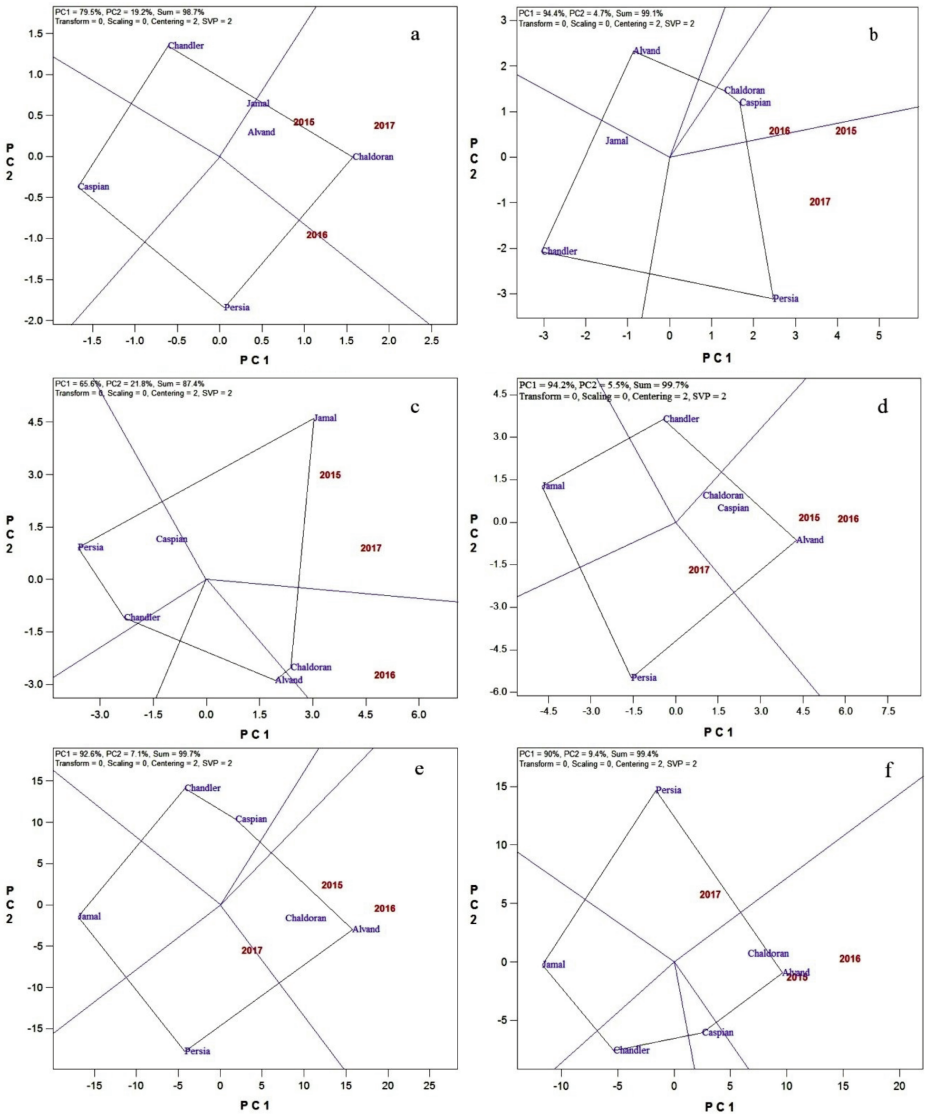

Fig. 3 - Genotype and Genetic × Environment (GGE) biplot of six walnut cultivars over three years (2015-2017) for nut weight (a), kernel percentage (b), fruit set percentage (c), nut number on scaffold cross area (SCA) (d), nut weight on SCA (e) and kernel weight on SCA (f).

and 99.4% of total variability. The GGE biplot for kernel weight on SCA was also divided into five sectors. The principal sector grouped the years 2015 and 2016, and the cultivars Alvand and Chaldoran with higher kernel weight on SCA. The second sector grouped the year 2017 and Persia that had greater kernel weight on SCA.

The figure 4 shows scattering of walnut cultivars based on the yield efficiency traits in a biplot. In figure 4b Chandler, Persia, Chaldoran and Alvand had the highest nut weight on SCA and also nut weight. The same results on figure 4 c with Chaldoran and Alvand which had the highest kernel weight on SCA too. According to figure 4 d the highest fruit set percent and nut weight on SCA also was belong to Alvand and Chaldoran.

Genetic and phenotypic correlations

The genetic and phenotypic correlations between the traits are reported in Table 3. These results showed that the nut weight did not considerably correlate with kernel percentage and number of nuts on SCA. Nut weight had a moderate impact on fruit weight on SCA (r_g = 0.31, r_p = 0.27) and kernel weight on SCA (r_g = 0.34, r_p = 0.29). As expected, a high genetic and phenotypic correlation was observed between the number of nuts on SCA and nut weight on SCA (r_g = 0.95, r_p = 0.95) as well as kernel weight on SCA (r_g = 0.90, r_p = 0.91) (Table 3). Kernel weight on SCA was significantly correlated with most of the traits, but the highest genetic and phenotypic correlations were observed between this trait and fruit weight on SCA (r_g = 0.97, r_p = 0.97).

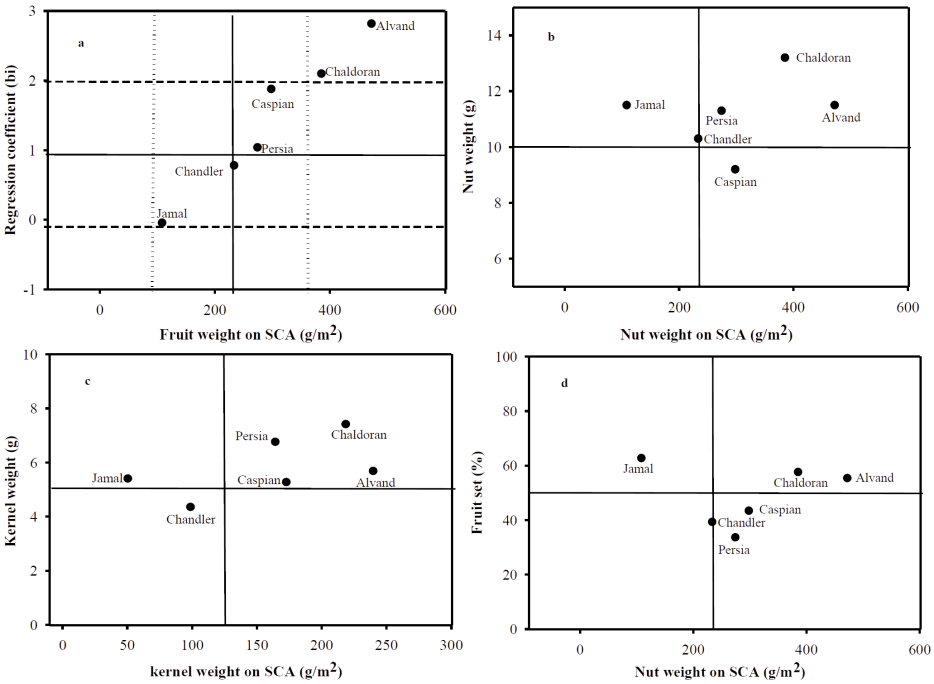

Fig. 4 - Biplot of regression coefficients against average yields of walnut cultivars (The horizontal solid line represents the mean coefficient of regression and the vertical solid line denotes the average fruit weight on scaffold cross area (SCA). The standard error (±1SE) was included and represented by the dotted lines for both yield efficiency and regression coefficients) (a), biplot of nut weight with nut weight on SCA (b), kernel weight with kernel weight on CA (c), and percentage of fruit set with nut weight on SCA (d). Solid lines in graphs show the average of each trait.

Table 3 - Genetic and phonotypic correlations of different traits

		Kernel (%)	Fruit set (%)	Number of nuts on SCA2	Nut weight on SCA	Kernel weight on SCA
Nut weight	G1	0.19	0.72	-0.02	0.31	0.34
	P1	0.12	0.53	-0.03	0.27	0.29
Kernel (%)	G	1	-0.34	0.25	0.31	0.55
	P		-0.26	0.26	0.30	0.52
Fruit set (%)	G		1	-0.29	-0.09	-0.14
	P			-0.12	0.03	0.01
Number of nuts on SCA	G			1	0.95	0.90
	P				0.95	0.91
Nut weight on SCA	G				1	0.97
	P					0.97
Kernel weight on SCA	G					1
	P					

G and P are the genetic and phenotypic correlations, respectively.
SCA = Scaffold cross area.

4. Discussion and Conclusions

The significant effects of year, genetic and genetic × year interaction showed that cultivar and its interaction with environmental conditions as the main determinants of cultivar's adaptability. Therefore, stable and compatible cultivars should be found and introduced for appropriate climate(s) (Rawandoozi *et al*., 2021). The low fruit set in 2017 caused the fruit production to be significantly lower compared to other two years, while there were no significant differences in pistillate flowers (data not shown).

Understanding the genotype and genetic × environment interaction also is important for increasing

the gain in cultivar improvement programs. The genotype's main effect nd especially it's G x E interaction implies the different performance of genotypes across environments that arises from the various sensitivities to the different environments (Rawandoozi *et al*., 2021). For all of the yield related traits, GGE biplot have described most of the existing variations (more than 90 %), which indicates the relative validity of the biplot in explaining the variations of genotypes and genetic × environment interaction (Yan and Tinker, 2006). The effect of genotype in determining the walnut yield efficiency has been demonstrated by Dogra *et al*. (2018). Based on our results high amounts of PC_1 has been recorded for kernel percentage (94.4%) as well as number and weight of nut and kernel on SCA (more than 90%). So, a high and stable production will be expected by selecting cultivars with higher yield efficiencies and more compatible with environmental conditions. The results on nut number and nut weight in cultivars are consistent with the findings of Mahmoodi *et al.* (2016). Fruit set is another important trait affecting the number of nuts in tree (McGranahan and Leslie, 2009; Sarikhani Khorami *et al.*, 2014; Khadivi-Khub *et al.*, 2015). It is clear that, number of pistillate flowers is relatively lower in cultivars with terminal bearing habit compared to lateral bearing ones (McGranahan and Leslie, 2009; Hassani *et al*., 2020 b). In terminal bearing cultivars like Jamal, the higher fruit set usually could compensate the production to some extent.

Among the yield related traits, the lowest value of the PC_1 was belong to fruit set (65.6%), which indicates this trait is more affected by genetic × environment interaction (21.8%). The results of this study present the clear effect of genotype on fruit set (Fig. 2 c) which is consistent with Kumar *et al.* (2005). Due to the fact that the amount of pistillate flowers produced in walnut is lower than pome and stone fruit trees, higher fruit set (50-90%) is necessary in order to produce an adequate yield. In addition to genetic, genetic × environment interaction plays a very important role in pollination and fruit set of cultivars (Cosmulescu *et al.*, 2010; Mariana and Sina Niculina, 2017). According to the results, there was a significant negative correlation between bud break and fruit set (R = -0.54), so that with delayed leafing, the fruit set decreased. It is clear that the late leafing cultivars deal better with late spring frosts (McGranahan and Leslie, 2006; Hassani *et al.*, 2013; Hassani *et al*., 2020 b), but the pollination and fruit set were not the same in late and early leafing walnut cultivars. To obtain a sufficient fruit set, care must be taken regarding producing a sufficient pollen volume with an adequate overlap of pollen-shedding for the receptivity period of pistillate flowers. The response of cultivars could be affected by different climatic conditions in different years especially at leafing time and time of pollination (Cosmulescu *et al*., 2010; Sarikhani Khorami and Vahdati, 2019; Cao *et al.*, 2020). Temperature is one of environmental factors influences the percentage of fruit set by influencing pollination factors, such as pistillate flowers receptivity and pollen-shedding period. In late leafing cultivars the environmental factors such as high temperatures at the pollination time, could lead to lower effective pollination period and pistillate flower receptivity period, that could reduce the fruit set (Ramos, 1997).

High broad-sense heritability relative to kernel weight and kernel percentage also indicated that these traits are less affected especially by genetic × environment interaction. Conversely, low-moderate heritability and high ratio of genetic × environment interaction for fruit set indicated substantial environmental effects on this trait. The heritability values in the present study were somewhat lower than what reported by Eskandari *et al*. (2006), Dogra *et al*. (2018), and Marrano *et al.* (2019).

In terms of yield stability, it seems that the genetics, environment and their interaction could contribute to various characteristics such as: fruit-bearing habit, growth vigor, nut weight, kernel weight, kernel percentage, previous year crop load, pollination, fruit set and late spring frosts (Cosmulescu *et al.*, 2010; Asma, 2012; Sarikhani Khorami *et al*., 2014; Dogra *et al*., 2018; Cao *et al.*, 2020; Hassani *et al*., 2020 a). Generally, in low-yielding cultivars such as Jamal, year-by-year variations of traits were low. However, they were higher in cultivars with more production such as Chaldoran and Alvand (Hassani *et al.*, 2020 a). Some studies have reported significant alternate bearing in walnut cultivars (Asma, 2012; Hassani *et al*., 2014; Mahmoodi *et al.*, 2016). So, alternate bearing, opposed to genetic stability, is affecting the fruit production trends of walnut cultivars in different years (Amiri *et al.*, 2010). Mahmoodi *et al.* (2015), reported the presence of 2-15% of alternate bearing among different walnut cultivars. In majority of high-yielding cultivars, a heavy crop load is followed by a low fruit production in the subsequent year. Asma (2012) found that the yield is influenced mostly by leafing time, fruit-bearing habit, tree

size, nut and kernel weights, and kernel percentage. Moreover, Dogra *et al.* (2018) reported that yield is controlled polygenically and is influenced by environmental conditions. They stated that pistillate flower density, fruit set, trunk section area, trunk circumference, tree height, shoot length, pollen-shedding period, fruit weight, kernel percentage, and shell thickness had affected the yield of walnut trees, which were in part consistent with the findings of the present study.

High variation was observed in yield components and yield efficiency traits with different environments and cultivars, and it was found that genetic by environment interaction are the most important factors determining yield variations. Understanding the contribution of genetics and genetic × environment interaction is very important. The genetic × environment interaction in fruit set was more than other yield-related traits, while the broad sense heritability (H^2_b = 0.36) was the lowest value. Therefore, fruit set is most affected by variation of environmental conditions, so that under undesirable climatic conditions it will be yield determining factor especially in late leafing walnut cultivars. Regarding the nut weight, the effect of genetic by environment interaction was strong while heritability was greatly affected by the environmental conditions. The contribution of genetic × environment interaction on other traits related to yield efficiency was estimated to be less than 10%. Genetic and phenotypic correlation also indicated that nut weight, kernel weight and kernel percentage had a low-moderate correlation with nut and kernel weight on SCA. On the contrary, the nut number on SCA had the highest genetic and phenotypic correlation with nut and kernel produced on SCA. The results showed that in walnuts, that is a nut tree species well adapted to temperate climate, the variability of yield related traits over environments (years), was highly significant. So, the change in climate in one hand and the scarcity of the resources (water and land) on the other hand emphasizes on more accurate evaluation on the new cultivars especially in yield related traits.

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Potassium silicate enhances drought tolerance of *Bellis perennis* by improving antioxidant activity and osmotic regulators

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Key words: Antioxidant activity, fertilizer, osmotic regulation, secondary metabolites.

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Abstract: Ornamental plants can usually encounter various types of environmental stress, which reduce plant productivity. A proper application of fertilizers can improve plants' tolerance to drought stress. Nutrients such as potassium and silicon are known to have beneficial effects. This study aimed to evaluate the growth of *Bellis perennis* under drought stress (80, 70, and 60% FC) and with the application of potassium silicate (0, 2, and 4 mM). The results showed that potassium silicate (2 and 4 mM) increased K and Si accumulation in plants under drought stress. Plants treated with potassium silicate under drought stress exhibited a lower degree of electrolyte leakage and less MDA accumulation in the following order: 2 and 4 mM potassium silicate. An increase in relative water content and chlorophyll was observed with application of potassium silicate under drought stress. Regardless of potassium silicate, the plant enzymatic defense system was significantly improved compared to non-stressed plants. Potassium silicate enhanced the amount of osmotic regulators (carbohydrate and proline) and secondary metabolites (flavonoids and phenols) compared to control plants regardless of drought stress. The anthocyanin content in the flowers significantly decreased by 32.2% when the plants were treated with 4 mM potassium silicate at 60% FC, compared to 80% FC. In conclusion, potassium silicate mitigated the effects of drought stress, enhanced plant tolerance to drought stress, increased the activity of antioxidant enzymes, and improved the amounts of osmotic regulators and secondary metabolites.

1. Introduction

Plant growth is usually affected by numerous abiotic stressors (Calanca, 2017). Deficits in water supplies are a major environmental threat, currently affecting more than 41% of the world's land mass. Projections for 2050 show a further increase in the magnitude and impact of this environmental threat (Prăvălie, 2016). As global temperatures increase, the annual maximum temperature is estimated to increase by 5°C per year by the end of the 22nd century. This problem will lead to

more frequent and extreme droughts in many parts of the world (Gao *et al*., 2020). Such environmental types of stress significantly cause a decline in crop yields falling below maximum potential (Raza *et al*., 2020). Environmental stress can suppress plant growth by interrupting various processes such as cell metabolism, nutrient uptake, and maintenance of turgor pressure (Kusvuran and Dasgan, 2017).

Regarding the proper use of fertilizers for improving plant tolerance to drought stress, the application of nutrients can be largely beneficial. Such applications have reportedly included potassium and silicon, with essential functions in plant metabolism (Bukhari *et al*., 2020; Ibrahim *et al*., 2020). Potassium is a vital fertilizer involved in numerous biochemical and physiological processes, including stress tolerance, plant growth, yield, and quality. Potassium (K) is necessary for many physiological processes, for example, maintaining turgor, translocation of photosynthetic substances to sinking organs, activation of enzymes, synthesis of proteins, transport of solutes in the phloem, and maintenance of cation-anion balance in the cytosol and vacuole. Furthermore, K reportedly facilitates osmoregulation, stomatal movements, and tropism, while it is mainly absorbed from the soil through the roots (Qi *et al*., 2019). However, drought stress causes a reduction in the absorption of elements. The reduced sensitivity of K-deficient plants to drought stress is related to several factors. These factors include the role of K in regulating stomata stomatal and water balance, as well as osmotic potential in vacuoles (Haworth *et al*., 2018). Applying potassium fertilizer mitigates the adverse effects of these stressors on plant growth (Qi *et al*., 2019).

Silicon (Si), the second most abundant element on Earth, can increase plant tolerance to biotic and abiotic stressors such as frost, heat, pests, drought, diseases, and nutrient imbalance (Wang *et al.*, 2021). Si deficiency reportedly decreased photosynthesis, also increased disease incidence, insect infestation, wilting, and postharvest decline. while all of these symptoms are signs of stress (Reynolds *et al*., 2009; Dallagnol *et al*., 2012; Weerahewa *et al*., 2015). Si usually contributes to healthy plant development and is essential for cell development and differentiation. The protective role of Si in drought conditions is mainly associated with an enhanced level of water retention, which promotes photosynthesis (Zhang *et al*., 2018). It accelerates the accumulation of osmolyte regulators (proline and carbohydrates) as well as antioxidant activities in plants exposed to stress in the environment (Moussa and Shama, 2019). Potassium silicate (K-silicate) is used as a source of highly soluble K and Si. K-silicate does not contain volatile organic compounds, and its application does not result in the release of hazardous or pollutant byproducts (Romero-Aranda *et al.*, 2006). In a relevant study, the application of K-silicate to the soil, for several plant species under irrigation with water-deficit conditions, resulted in the highest biomass of all species (Moussa and Shama, 2019). Si can act as a growth regulator and can potentially increase plant growth under drought stress. Spraying K-silicate and other nanomaterials can potentially reduce the adverse effects of drought stress on crops (Zahedi *et al*., 2020).

The common daisy (*Bellis perennis*) belongs to the Asteraceae family and is known as an archetype. *Bellis perennis* is an important ornamental and medicinal plant with a global distribution. It is one of the first flowering species and is a crucial member of spring bloomers (Siatka and Kašparová, 2010). Daisies are grown for their beauty, either for color or aesthetic reasons. They are naturally able to provide economic, environmental, and social benefits. However, water-deficit largely affects the ornamental value of this species.

In the current study, we focused on the response of daisies to drought stress, while monitoring their antioxidant enzymes, substances for osmotic regulation, and secondary metabolites. Although many studies have already considered the role of potassium silicate in reducing the adverse effects of drought stress in various plants, there is little information about the effects of potassium silicate on ornamental plants under drought-stress conditions. Therefore, the objective of the current study was to investigate the effects of potassium silicate on the characteristics of daisies under drought-stress conditions. The mechanisms of action by potassium silicate, their advantages for ornamental plants, and their ability to create drought tolerance provide a scientific basis for using potassium silicate to alleviate drought stress.

2. Materials and Methods

Plant culture, drought stress, and potassium silicate treatments

This study was carried out at the Ferdowsi University of Mashhad (autumn-spring 2021). Seeds of *Bellis perenesis* L. were purchased from Takii seed

company. In September 2021, the seeds were grown in polyethylene bags containing a mixture of peat and perlite (3:1) for four weeks (trifoliate stage) under controlled conditions (21°C/17°C day/night and 45-55% humidity under 100 mmol photons m^{-2} s^{-1}. Four weeks later, the trifoliate seedlings were transplanted into pots. For each treatment, the experiment was laid out with three pots. The pots were placed in a greenhouse (air temperature of 21±2°C and relative humidity of 62±2%) during the growing periods. Irrigation started after one day, and the plants were well watered for a few months (90-85% FC). Drought treatment was initiated by omitting irrigation, and potassium silicate (K_2SiO_3) was used as the Si source. Potassium was administered to the plants in the form of liquid potassium silicate (K_2SiO_3) (10% K_2O, 25% SiO_2) at three concentrations (0, 2, 4 mM) (The concentrations of potassium silicate were selected according to a pretest). In March, the plants were treated with different solutions, i.e. (1) 1/2 Hoagland's solution without the addition of K_2SiO_3, (2) 1/2 Hoagland's solution with the addition of 2 mM K_2SiO_3, (3) 1/2 Hoagland's solution with the addition of 4 mM K_2SiO_3. Potassium silicate was applied as a treatment for one month in March, and irrigation treatments began with drought stress (80%, 70%, and 60% FC) in April. Three levels of water deficit (i.e. 80, 70, and 60% of field capacity, FC) were applied from April to June. The gravimetric method (Campbell and Mulla, 1990) was used for irrigation for two months. First, several pots were completely irrigated so that the water permeated all pores in the soil. Then, the pots were wrapped with plastic covers to prevent evaporation and transpiration. The pots were weighed until their weight remained constant for two consecutive measurements. Then, a soil sample was taken to the laboratory. The fresh weight was measured and the dry weight was calculated after 12 hours of storage in an oven at 105°C. The percentage of moisture content by weight, required for suitable crop production, is calculated based on the following equation:

$$FC = (A-B / B) \times 100$$

where FC, A, and B are the field capacity, the weight of moist soil after gravity drainage, and the weight of the sample dried at 105°C for 12 hours, respectively.

The weight difference between water-saturated and oven-dried soil was taken as the weight of water needed to bring the pots to field capacity, and then lower water contents in the soil (% field capacity) were calculated accordingly. During the period of treatments, the pots were regularly weighed, and additional water was supplied when necessary. For each test, there were three replicates containing five plants, making a total of 15 plants. At the end of the experiment, the fresh leaves were used for measuring electrolyte leakage, RWC, and chlorophyll content. For determining the nutrition concentration and proline content, dried leaves were frozen in liquid nitrogen and stored at -80°C until the time of measurements.

Determination of K and Si concentration

The K and Si concentrations were determined on the dry leaves samples. Oven-dried leaves (300 mg of the dried samples) were weighed and burned in a muffle furnace at 550°C for 8 hours. The K concentration was determined by flame photometry (PFP7, Jenway, UK). The Si concentration was determined by the colorimetric ammonium vanadate method (Jaiswal, 2003).

Measurement of electrolyte leakage and relative water content

Electrolyte loss was determined according to a method used by Gusta *et al.* (2003), and relative water content (RWC) was calculated via a method used by Pieczynski *et al*. (2013).

Measurement of antioxidant activity and malondialdehyde (MDA)

Antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The extract (100 mg of fresh weight + ethanol) was blended with 960 µL of DPPH in methanol. The supernatant was centrifuged for 5 min and kept in the dark room. The DPPH was determined using a Shimadzu UV-1800 spectrophotometer at 515 nm (Kedare and Singh, 2011). Malondialdehyde (MDA) content was measured according to Velikova *et al.* (2000) methods. Leaf tissues (0.5 g of each) were homogenized in 8 ml of 0.1% (w/v) trichloroacetic acid and the homogenates were centrifuged for 10 min at 4 °C, after which the supernatants were used for malondialdehyde analysis. Equal volumes of extracts were mixed with 0.5% (w/v) of thiobarbituric acid made in 5% (w/v) trichloroacetic acid and heated at 100°C water bath for 20 min, after which their actions were stopped in the ice bath. After centrifuging,the absorbance of the supernatant was measured at 450, 532, and 600 nm.

Determination of antioxidant enzyme activities

Extraction was performed according to a method used by DaCosta and Huang (2007). Samples (0.5 g of fresh weight) were ground in liquid nitrogen and were homogenized in 4 mM phosphate buffer (pH 7.8), 60 M riboflavin, 195 mM methionine, 3 M EDTA, and 1.125 mM nitro blue tetrazolium chloride (NBT). Enzyme activities were expressed per fresh weight of the sample. One SOD activity unit was defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium chloride (NBT) photoreduction (Sairam *et al.,* 2002). Catalase activity (CAT) was measured as described by Abedi and Pakniyat (2010). The reaction solution consisted of 50 mM K-phosphate buffer (pH 7.0), ten mM H_2O_2, and 50 mL enzyme extract. The decomposition of H_2O_2 was measured at 240 nm. The peroxidase activity (POD) was measured by the guaiacol method (Guan *et al*., 2015). The oxidation of guaiacol was monitored by observing changes in the absorbance values at 470 nm for 3 min. The reaction mixture contained 50 ml of 100 mM PBS (pH 6.0), 10 mM H_2O_2, 2.58 mM of guaiacol. The reaction was started by adding the enzyme extract to the reaction mixture solution.

Measurement of photosynthetic pigments

Chlorophyll contents (Chl a, b, and total Chl) were measured by squashing the leaves (200 mg) in 10 ml 80% acetone solution, and the chlorophyll content was determined at 645 and 663 nm, respectively using a Shimadzu UV-1800 spectrophotometer (Nagata and Yamashita, 1992).

Determination of osmotic regulators

Carbohydrates were determined using the Anthrone reagent method. Fresh leaves (500 mg) were placed in 70% methanol and reached the required volume with distilled water. The samples were used for estimations of carbohydrate content using the Anthrone reagent (McCready *et al.,* 1950). Proline content was calculated according to Bates *et al.* (1973). The leaf extract (0.1 mg leaf sample + 10 ml sulfosalicylic acid) was homogenized in glacial acetic acid and ninhydrin acid. Then, the solution was heated in a boiling water bath. After cooling, 5 mL of toluene was added, and then the top layer of the solution was removed and centrifuged at 3000 g for 5 minutes. The proline content was determined at 520 nm using a Shimadzu UV-1800 spectrophotometer.

Secondary metabolite measurements

Total phenolic content was measured using the Folin-Ciocalteu reagent method (Singleton and Rossi, 1965). In the Folin-Ciocalteu method, 250 µl of the alcoholic extract (100 mg + 10 ml ethanol) was diluted to a known volume with distilled water, 10% Folin reagent, and 7.5% sodium carbonate. The phenolic content was determined at 675 nm using a Shimadzu UV-1800 spectrophotometer. Assaying the total anthocyanin content followed, a method by Sukwattanasinit *et al.* (2007), where two buffer solutions were used (25 mM K-chloride pH 1.0 and 0.4 M Na-acetate pH 4.5). The values were noted at 510 nm using a Shimadzu UV-1800 spectrophotometer. Flavonoid content was assayed according to a method by Zou *et al*. (2004). The extract (500 mg + 5 ml ethanol) was homogenized in 4.5 mL distilled water and 0.3 mL 5% $NaNO_2$. Next, after mixing the solution properly, 1 mL of 10% $AlCl_3$-$6H_2O$, 2 mL of 1 M NaOH, and distilled water were added to the reaction mixture. The absorbance values were determined at 510 nm using a spectrophotometer (Shimadzu UV-160A).

Statistical analysis

The difference between treatments was determined using a factorial layout and a completely randomized experimental design with three replicates followed by the LSD testing ($P<0.01$). Data were subjected to two-way analysis (ANOVA) with repeated measures and were analyzed using the SAS statistical package (version 9.2, SAS Institute, Cary, NC, USA).

3. Results

The potassium and silicon concentrations were significantly ($P<0.01$) affected by fertilizer and drought stress. Potassium (K) concentration in the control plants decreased by 14.9% under drought stress at 60% FC compared to 80% FC. A decrease in leaf K content was observed by the effect of potassium silicate at a concentration of 2 and 4 ppm by 8.08 and 21.2%, respectively, under 70% FC. However, the amount of decrease was 8.7 and 18.2%, respectively, under 60% FC (Fig. 1 a). The Si concentration was significantly improved by all potassium silicate applications under the water deficit conditions. The Si concentration increased in response to 4 mM potassium silicate under 80 and 60% FC (by 926 and 998%, respectively) compared to control plants (Fig. 1 b).

The results showed that the interaction of potassium silicate and drought stress significantly ($P<0.01$) affected electrolyte leakage, RWC, antioxidant activity, and MDA accumulation. Potassium silicate signifi-

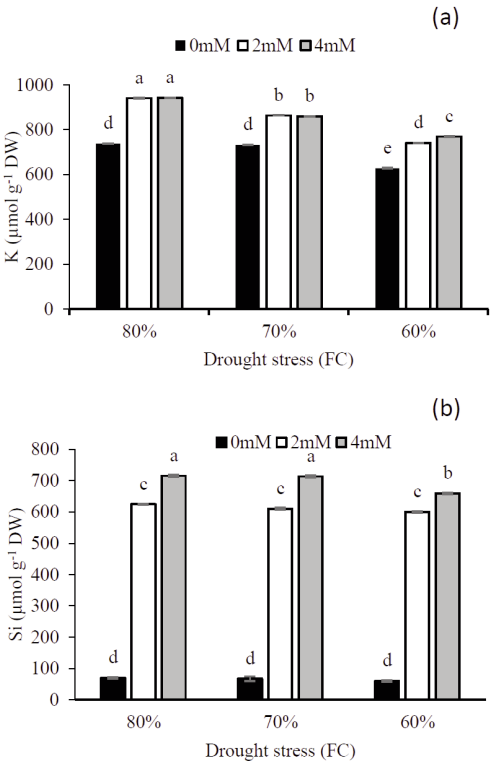

Fig. 1 - Effect of exogenous application of potassium silicate on K (a), and Si (b) of daisy under water-stress conditions. Bars with a different letter differ significantly (P<0.05) according to the LSD test.

cantly inhibited the decrease in electrolyte leakage, whereas a more significant level of decrease was observed in the control plants at 60% FC. As shown in figure 2a, electrolyte leakage was increased by 102, 150, and 220% at 60% FC in control plants, 2 and 4 mM compared to 80% FC, respectively. RWC was reduced by 8.6 and 11.7% under drought stress (60% FC) in response to 2 and 4 mM potassium silicate compared to 80% FC. Compared with the control, the application of 4 mM potassium silicate significantly increased the RWC of plants, whereas 2 mM potassium silicate had no significant effect on the RWC compared to the control plants (Fig. 2 b).

The antioxidant activity increased significantly in response to drought stress, but the application of 2 and 4 mM potassium silicate under severe drought stress resulted in even higher values of antioxidant activity. Nonetheless, no significant difference was observed between potassium silicate-treated plants and the control plants at 80% FC. The antioxidant activity reached maximum values, increasing by 33.7 and 36.5% when the daisies were treated with 2 and 4 mM potassium silicate at 60% FC compared to the control plants, respectively (Fig. 2 c). The role of potassium silicate at 2 and 4 ppm was effective in reducing MDA accumulation under drought stress. Plants treated with potassium silicate under drought stress showed lower MDA levels in the following order: 2 and 4 mM of potassium silicate than control

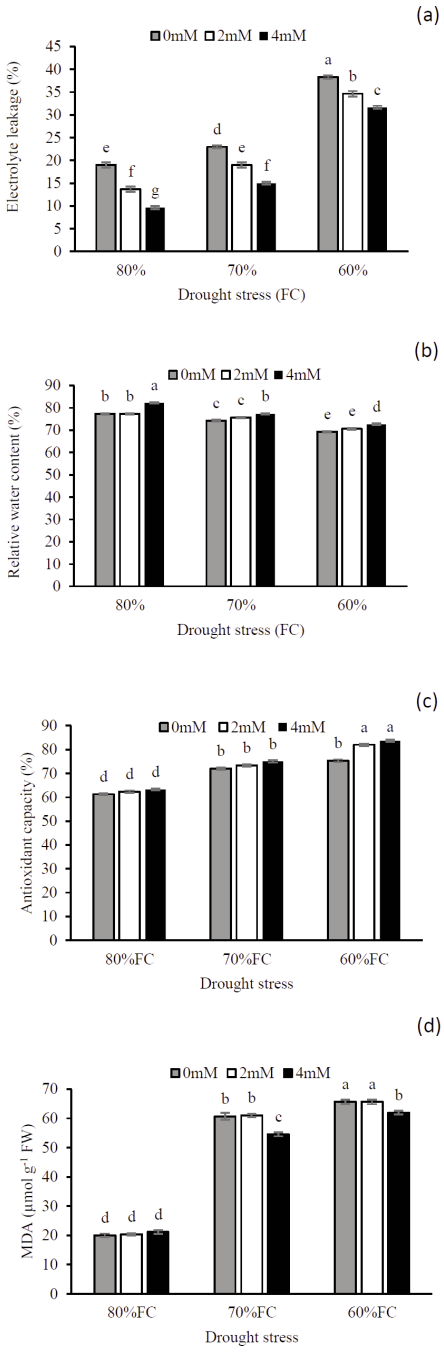

Fig. 2 - Effect of exogenous application of potassium silicate on electrolyte leakage (a), relative water content (b), antioxidant activity (c), and MDA (d) of daisy under water-stress conditions. Bars with a different letter differ significantly (P<0.05) according to the LSD test.

plants. Thus, decreasing effect on this trait resulted from using 4 mM potassium silicate at 70 and 60% FC. The MDA peaked when the daisies were treated with distilled water and 2 mM potassium silicate at 60% FC. However, no significant difference was observed between the control plants and either of the 2 and 4 mM potassium silicate treatments under the effect of 80% FC (Fig. 2 d).

Drought stress significantly (P<0.05) increased the activities of antioxidant enzymes, catalase, peroxidase, superoxide dismutase, and aspartate peroxidase by 7.76-31.54%, 313-323%, 127-181%, and 4.22-99.35%, respectively, under drought stress (Fig. 3). In general, potassium silicate increased all of the mentioned enzyme activities, but this increase was higher in reponse to the 4 mM treatment under drought stress. The application of potassium silicate at 2 and 4 mM significantly improved the activity of CAT by 29.4 and 35.2%, respectively, in daisies grown at 60% FC compared to the control plants (Fig. 3 a). All potassium silicate treatments significantly improved the activity of POD under drought stress conditions. The POD activity increased in response to the 70% FC compared to the control, but decreased more at 60% FC, compared to 70% FC. As shown in figure 3 b, the activity of POD increased by 42.7 and 50.3% in plants treated with 2 and 4 mM potassium silicate, respectively, compared to the control plants under 60% FC.

Under the conditions of drought stress, the application of potassium silicate significantly increased the activity of APX. In response to 60% FC, the plants showed the highest APX activity. Although no significant differences were observed between potassium silicate-treated and control plants at 60% FC, a sharp increase in APX activity was observed when 4 mM potassium silicate was used along with drought stress. The application of 4 mM potassium silicate increased the activities of APX by 14.5% at 80% FC and by 87.8% at 60% FC compared to the control plants (Figs. 3c). The application of potassium silicate increased the SOD activity under drought stress conditions. This pattern of increase was more prominent (37.5 and 30.2%) at both potassium silicate levels along with moderate drought stress, compared to 80% FC, whereas it was least prominent in severe conditions (60% FC). The activity of SOD in daisy leaves increased by 28.4 and 21.5% at 60% FC, using potassium silicate at 2 and 4 mM, respectively, compared to well-watered plants (Fig. 3 d).

The data revealed that the chlorophyll (chl) a, b, and total chlorophyll contents were significantly (P<0.05) affected by fertilizer and drought stress. Regarding chl a, b, and total chl in the leaves under drought stress, these parameters decreased in response to the drought stress severity . This downward trend was 34.68 and 55.4% higher in the case of Chl a, but was 2 and 6.25% lower in the case of Chl b when severity of drought increased. The application

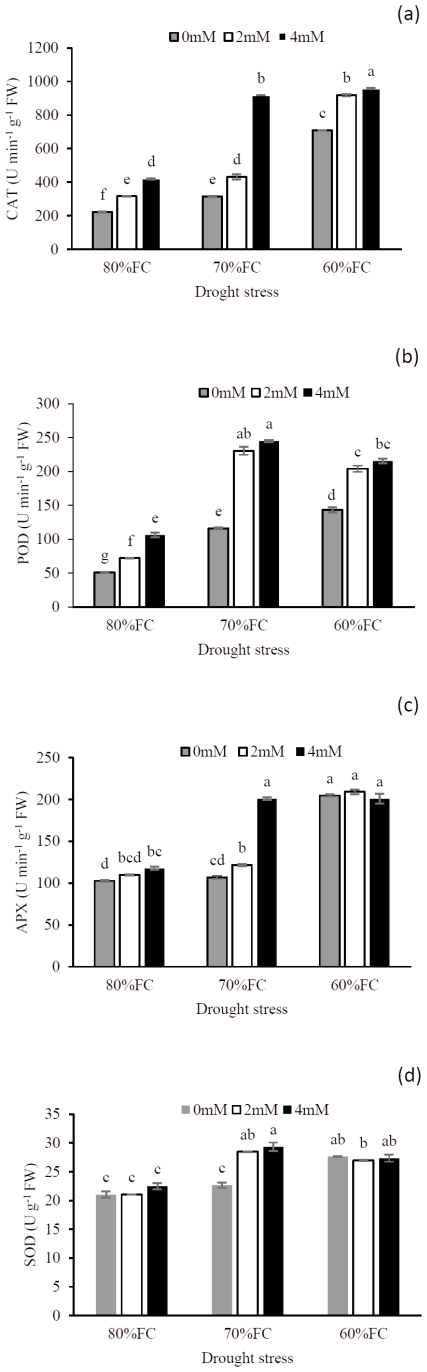

Fig. 3 - Effect of exogenous application of potassium silicate on CAT (a), POD (b), APX (c), and SOD (d) of daisy under water-stress conditions. Bars with a different letter differ significantly (P<0.05) according to the LSD test.

of potassium silicate at a concentration of 2 mM increased the chl a and chl b by 68 and 67%, respectively, at 70% FC. Furthermore, the mentioned values were increased by 41.4 and 97%, respectively, at 60% FC compared to the control. The highest content of chl a and b were observed when plants were treated with 4 mM potassium silicate at 80 and 70% FC (Figs. 4 a, b). During drought stress, total chl gradually decreased in response to greater intensity of drought stress. The total chl value decreased by 127% under 60% FC compared to 80% FC and by 98% compared to 70% FC. This value was also affected by potassium silicate under drought stress. Total Chl content was significantly improved by all potassium silicate applications under the drought stress conditions. Total chl content increased by 7.4 and 72% in response to 4 mM potassium silicate at 80 and 70% FC, respectively (Fig. 4 c).

The application of potassium silicate significantly (P<0.05) increased the amount of carbohydrate and proline content in the leaves under drought stress. At 80% FC, potassium silicate-treated plants had no significant difference from the control plants in terms of carbohydrate content. By applying drought stress, an increase in carbohydrates was observed in the leaves when the plants were treated with potassium silicate. In response to 2 and 4 mM potassium silicate, the carbohydrate content increased by 31.3% and 26.6% at 70% FC, and by 60.8% and 53.2% at 60% FC, respectively, compared to the 80% FC (Fig. 5a). Regarding proline changes in the leaves, there was an increase of 4.7- and 1.6-fold by the effect of 60% FC and 70% FC, respectively, compared to the 80% FC. The application of 2 and 4 mM potassium silicate increased this parameter further by 75 and 42% at 70% FC, respectively, but by 385 and 312% at 60% FC, compared to the control plants. The highest proline

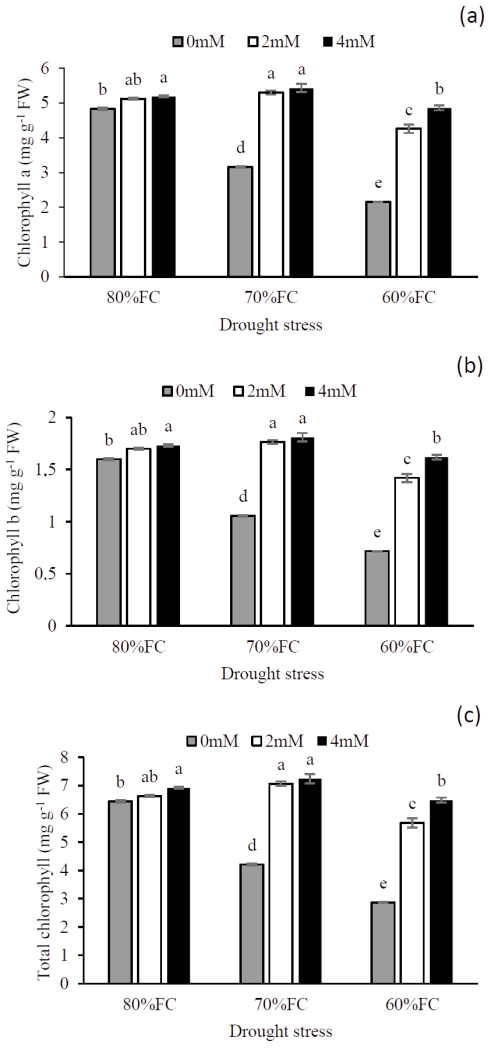

Fig. 4 - Effect of exogenous application of potassium silicate on chlorohyll a (a), chlorohyll b (b), and total chlorohyll (c) of daisy under water-stress conditions. Bars with a different letter differ significantly (P<0.05) according to the LSD test.

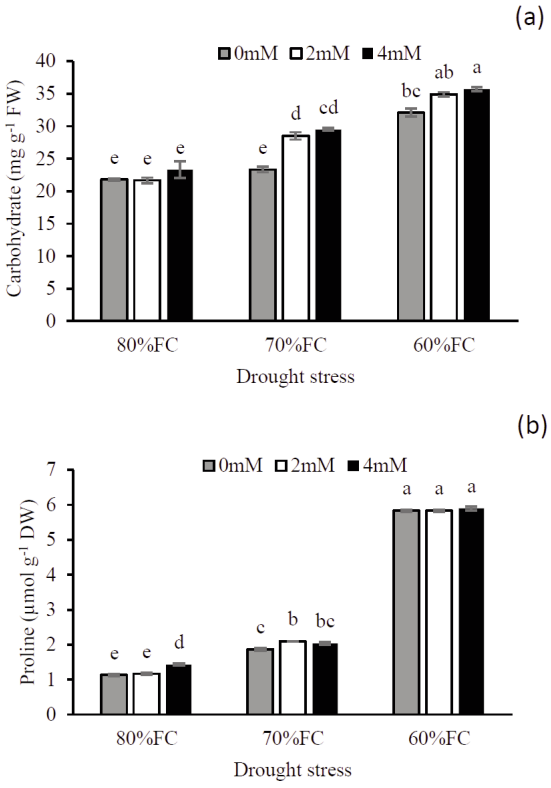

Fig. 5 - Effect of exogenous application of potassium silicate on carbohydrate (a), and proline (b) of daisy under water-stress conditions. Bars with a different letter differ significantly (P<0.05) according to the LSD test.

content was observed in drought-stressed plants (60% FC) treated with potassium silicate. However, no significant difference was observed between 2 and 4 mM potassium silicate under severe drought stress (Fig. 5b).

In general, potassium silicate (2 and 4 mM) at 80% FC significantly (*P<0.01*) increased the flavonoid content by 36 and 45.5% compared to the control plants. In the control plants. Increasing the severity of drought stress at 70 and 60% FC decreased this trait by 50.2 and 97.2%, respectively. The application of 2 and 4 mM potassium silicate increased the flavonoid content by 10.3 and 41.9%, respectively, at moderate water deficit (70% FC), but by 51.7 and 62.3%at severe water deficit (60% FC), respectively, compared to the 80% FC (Fig. 6 a). Figure 5b shows that the phenolic content increased in response to the intensity of drought stress, and the highest value was found at 60% FC. Total phenolic content increased significantly by 31.1 and 43.8% when potassium silicate-treated plants (4 mM) were under the effect of 80 and 70% FC, respectively, compared to the control plants. Potassium silicate (2 mM) increased the phenolic content by 27 and 46%, respectively. At 4 mM, it caused an increase of 39 and 57%, respectively, under the effect of 70 and 60% FC, compared to 80% FC. The highest anthocyanin content occurred when the potassium silicate-treated plants were grown at 80% FC. As for anthocyanin changes in the flowers, there was a decrease in response to the intensity of drought, but potassium silicate increased the value further. The anthocyanin content decreased in response to 4 mM potassium silicate at 60% FC, compared to 80% FC (11.2%). Both concentrations of potassium silicate significantly increased the anthocyanin content in the flowers by 228% at most, under the effect of 80% FC, compared to the control plants (Fig. 6 c).

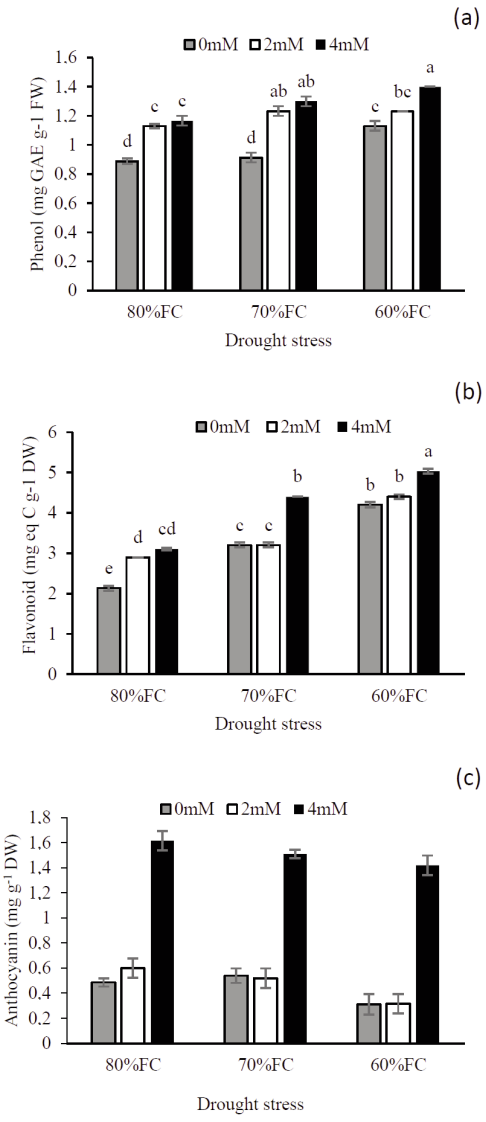

Fig. 6 - Effect of exogenous application of potassium silicate on phenol (a), flavonoid (b), and anthocyanin (c) of daisy under water-stress conditions. Bars with a different letter differ significantly (P<0.05) according to the LSD test.

4. Discussion and Conclusions

The application of potassium silicate improved nutrient uptake under drought stress. Si is not an essential nutrient but protects plants from a variety of biotic and abiotic stresses (Ranjan *et al.,* 2021). The highest concentration of K and Si in the plant occurred in response to 4 mM potassium silicate at 80% FC. These results emanate from the ability of plants to enhance root growth after potassium and silicon application, thereby increasing nutrient uptake (Sustr *et al*., 2019). The increase in K uptake is usually concomitant with a decrease in plasma membrane permeability and an increase in Si-induced H-ATP activity in plasma membranes (Zhu and Gong, 2014). K deficiency decreases the uptake and transfer of some nutrients by inhibiting enzymatic activities such as synthases, transferases, and kinases (Liu *et al*., 2013). Si leads to more significant root activity, consequently, a greater amount of nutrient uptake. Sarto *et al*. (2014) attributed the beneficial effects of Si to its concentration in the leaves and stems of wheat. Si is also known to influence the development of apoplastic barriers in roots by controlling apoplas-

tic pathways, followed by its translocation through the root apoplast to the shoot (Vaculík *et al*., 2012). One explanation for the increased tolerance in plants growing under water-deficit conditions could be a decrease in transpiration through the stomata and cuticle due to Si application. Silicon not only affects nutrient availability and uptake but also nutrient translocation from the roots to the shoots (Greger *et al*., 2018). In this study, drought significantly decreased the concentration (%) of K and Si. Also, drought stress can have a significant impact on plant nutrient ratios. Several studies have shown that drought can reduce nutrient uptake from the soil (Ge *et al*., 2012; Bista *et al*., 2018).

Under water-deficit conditions, potassium silicate reduces electrolyte leakage. Potassium silicate plays an important role in plant resistance to environmental stress. Within the plant, silicate is an immobile element that becomes a polymer gel and reduces the loss of ions from biomembranes after being deposited in the cell. The results of this study are consistent with a previous study by Othmani *et al.* (2021) who found that the more significant stability of the cell membrane in the presence of silicon was due to the hardening and strength of the cell wall. The water content of leaves under drought stress (70 and 60% FC) decreased compared to the 80% FC. Drought stress reportedly caused a decrease in leaf water content in most plants (Santos *et al*., 2021). In our study, the administration of potassium silicate led to an increase in the relative water content of leaves. A lower rate of water loss in silicate-potassium-fed plants can also be attributed to the lower transpiration of the plants. The accumulation of silicate in the lower epidermal cells reduces water loss through the cuticle. Si is deposited in plant tissues in the apoplast of the cell wall to form silica, thereby maintaining tissue integrity (Guerriero *et al*., 2016). In addition, potassium is primarily an important osmotic regulator in plants. Between 30 and 50% of the osmotic potential of leaf tissue is regulated by K (Turcios *et al*., 2021).

In the current study, potassium silicate reduced plant injury by decreasing MDA and increasing antioxidant activity. Malondialdehyde is the peroxidation product of unsaturated fatty acids in phospholipids. Therefore, the production of malondialdehyde under stress conditions can be used as a marker of lipid peroxidation (Ayala *et al*., 2014). As a result of drought, the peroxidation of glycopeptides occurred in chloroplast thylakoid, followed by the formation of diacylglycerol, triacylglycerol, and free fatty acids, leading to an increase in malondialdehyde in plant tissues (Sofo *et al*., 2004). Fatty acids and lipids are reportedly sensitive to oxygen species and are rapidly oxidized. The results are in line with previous studies indicating a positive effect of potassium silicate on malondialdehyde levels in damask rose (*Rosa damascena* Miller) under drought stress (Farahani *et al*., 2020). The ability of plants to scavenge free radicals was impaired by both drought and the addition of potassium silicate compared to the control plants. DPPH inhibition levels were below 70% and 60% FC at 4 mM potassium silicate compared to the control. Under drought stress, DPPH levels increased, and potassium silicate further enhanced the DPPH levels (Zahedi *et al*., 2020).

In this study, the administration of potassium silicate under drought stress conditions (i.e. the application of irrigation water to maintain 70 and 60% FC) increased all antioxidant enzyme activities. An increase in antioxidant activity in the leaves occurred in response to both potassium silicate concentrations and drought stress. The high activity of antioxidant enzymes such as CAT, POD, APX, and SOD in plants is an adaptive mechanism that protects cells from oxidative damage by reducing the concentration of hydrogen peroxide generated by cellular metabolism (Jan *et al*., 2022). Improving potassium concentration leads to an increase in photosynthetic products, the control of ionic balance, osmotic regulation, and an increase in enzymatic activity. By stimulating the activity of POD and APX through the detoxification of hydrogen peroxide, Si prevents oxidative stress and inhibits the production of hydroxyl radicals (Kim *et al*., 2017). In agreement with the current results, Ahmad *et al*. (2019) reported that using silica on mung beans (*Vigna radiata* L.) increased catalase and superoxide dismutase activities under drought stress. Superoxide dismutase is an enzyme that converts superoxide free radicals into hydrogen peroxide and oxygen while playing an important role in protecting cells from the negative effects of free radicals. SOD is the first line of defense of cells against free radicals under stress conditions (Ighodaro *et al*., 2018). The effects of Si nutrition on SOD activity and free radical elimination have been reported in the available literature (Geng *et al*., 2018). Gong *et al*. (2005) reported that using potassium silicate increased the activity of antioxidant enzymes in wheat (*Triticum aestivum* L.) under drought stress. The removal of reactive oxygen species decreases cell membrane permeability and

increases the activity of catalase, peroxidase, and superoxide dismutase, which indirectly decrease cell membrane lipid peroxidation and reduce the amount of malondialdehyde (Sharma *et al*., 2012).

The application of potassium silicate along with drought stress increased the amounts of photosynthetic pigments. A decrease in chlorophyll content occurred due to drought stress and was accompanied by an increase in the production of oxygen radicals in the cells. The radicals usually cause peroxidation, and consequently, the degradation of photosynthetic pigments. The effect of potassium silicate on the stability of plant pigments usually results from the accumulation of silicate in the epidermal cells, which has an indirect protective effect on the photosynthetic establishments, thereby reducing the stress-induced damage to photosynthetic pigments. Similar to the current results, a moderating effect of potassium silicate was reportedly observed on the chlorophyll content of *Rosmarinus officinalis* L. plants (Waly *et al*., 2019).

The results showed that the concentration of osmotic regulators (i.e. proline and total carbohydrates) increased significantly when potassium silicate was applied under water-deficit conditions. Silicon and potassium increase the production of carbohydrates and proline by increasing the osmotic potential, possibly through the accumulation of free radicals produced by the plant. They are thought to play an adaptive role in mediating osmotic adjustment and protecting subcellular structures in stressed plants (Hajiboland *et al*., 2017). These effects suggest that potassium and silicon may enhance leaf osmotic potential by converting starch to soluble sugars, especially under severe drought stress (Zahoor *et al*., 2017). It appears that potassium silicate stimulates carbohydrate production and, thus, alters the metabolism of plant-absorbed K and its conversion to proteins (Hafez *et al*., 2021). Si can directly or indirectly induce the biosynthesis of proline. Garg and Sing (2018) showed that the application of Si increased the activity of pyrroline-5-carboxylate synthetase (P_5CS) and glutamate dehydrogenase (GDH). In addition, the increase in proline because of potassium silicate treatment may highlight the importance of potassium and silicon in protecting cell membranes and maintaining relative water content under inadequate irrigation conditions. In this context, using silica on borage (*Borago officinalis* L.) plants reportedly increased the amount of proline in the leaves (Gagoonani *et al*., 2011). In agreement with these results, Ibrahim et al. (2020) reported that potassium silicate increased the proline content of maize plants under drought stress.

By reducing vegetative growth and altering the anatomical structure of the plant through the induction of secondary stress, e.g. oxidative stress, the effect of drought stress usually cause changes in the pathways of synthesis that make secondary compounds and metabolites (Ahanger *et al*., 2017). Polyphenols can improve plant tolerance to drought stress and play an important role as a carbon sink at times of stress. These effects may explain significant improvements in total phenolics in daisies because of their exposure to drought stress (Fig. 6 a). The increase in total soluble phenols in response to the application of K_2SiO_3 under drought stress could be a supporting effect of Si, thereby increasing plant tolerance, especially under water-deficit conditions. Fouda *et al*. (2021) found that potassium silicate increased the total flavonoid content in field beans. Feeding plants with Si and K-containing compounds has reportedly resulted in changes in the expression pattern of many genes. In particular, feeding plants with potassium- and silicon-containing compounds has led to changes in genes that encode enzymes involved in the phenylpropanoid pathway (Wang *et al.,* 2017). Indeed, the increase in phenylalanine ammonia-lyase activity is a common feature in plants treated with silicon, thereby enabling an increase in the synthesis of phenolic compounds. Potassium silicate increases polyphenols in many plants by activating enzymes that are relevant to the phenol production pathway, such as the phenylalanine ammonia-lyase (Vega *et al*., 2019).

The ability of plants to tolerate drought could be mainly explained by an increase in flavonoid content since flavonoids are compounds with strong antioxidant activity. Perin *et al*. (2019) suggested that the relationships between ABA metabolism, phenylpropanoid, flavonoid, and anthocyanin pathways can reduce drought stress. Probably, this could be one of the main reasons for the better tolerance of plants to drought stress. Drought largely affects the average performance of plant traits by reducing their properties, leading to a decrease in the associated anthocyanin content. Under drought stress, the anthocyanin content decreased, but potassium silicate increased the anthocyanin content of flowers (Fig. 6 c). These results are consistent with a previous study by Cirillo *et al*. (2021) in which anthocyanin content was reduced by the effects of stress. Jafari *et al*. (2015) reported that silicon treatment under osmotic

stress significantly increased the amount of non-enzymatic antioxidants (e.g. anthocyanins, flavonoids, and total phenolic compounds) and nutrients (Si, K^+, and Ca^{+2}) in cucumber plants.

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Yield performance and nutritional quality of tomato hybrids in response to protected environments during the Amazonian summer

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Key words: Carotenoids, fruit production, physicochemical properties, protected cultivation, *Solanum lycopersicum* L.

Abstract: This study evaluated the yield performance of the tomato hybrids 'DS0060', 'Thaise' and 'Trucker' in the open field and environments protected by agricultural film (F) and polycarbonate panels (P) during the Amazonian summer. In the protected environment, the crops produced significantly higher yields than in the open field. 'Thaise' has high thermotolerance and is adaptable to a wide temperature range, making it the best-performing hybrid in environment F. Highest yields were found for 'Thaise' in environment F or P (86.2 and 92.5 t ha^{-1}) together with 'DS0060' and 'Trucker' in environment F (75.3 and 88.2 t ha^{-1}), demonstrating the high yield potential in the interim growing season (January to April). In the open field, the fruit color was paler, fruit flesh firmer and ripening index lower. In environment F, the fruits contained highest levels of soluble solids, lycopene and β-carotene. 'Thaise' contained higher concentrations of these two compounds. Under environment P, the yield of the evaluated tomato hybrids increased considerably, indicating it as a promising possibility for tomato cultivation in tropical regions. 'Thaíse' stood out with high yield and good quality traits, when grown in an F or P environment. These results prove the viability of tomato production as interim crop in tropical regions, under high rainfall and heat, as well as the difference protected environments make for tomato cultivation, in particular the choice of the most suitable cover material for the crop, to ensure high yields coupled with desirable quality properties.

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is the fruit vegetable crop for which the market demand is the highest in the world. In 2019, the crop acreage was 5 million hectares, for a production of 181 million tons. Worldwide, Brazil is the 9th largest producer, with an output of 3.6 million tons on 54,500 hectares (FAO, 2021). The high demand for tomato is related to the palatability, culinary versatility and high contents of nutrients, especially those with functional properties such as vitamin C, lycopene and β-carotene (Ali *et al.*, 2021).

In tropical farming, tomato is considered a high-risk crop since the investments of inputs and management required are high. Open-field yield can vary considerably due to pest and pathogen pressure, since up to 75% of the plants may be affected from the very beginning of the season, mainly by bacterial wilts and viruses (Huat *et al.*, 2013). Protected cultivation allows production under adverse conditions, reducing plant exposure to high rainfall and, consequently, to disease incidence (Bazgaou *et al.*, 2018). By minimizing the seasonality effect, year-long production becomes possible, favoring product supply between the main crop seasons.

The cover material for a protected environment must be chosen with a view to reducing the levels of global radiation and incident photosynthetically active radiation (PAR), to ensure an optimized plant performance. Agricultural film can increase plant production by altering the levels of luminosity, humidity and air temperature (Beckmann *et al.*, 2006). In tropical regions however, it can cause a rise in air temperature of 10 to 12°C with flower and fruit dropping, fruit cracking, black spot, and a decline in lycopene synthesis and marketable fruit yield. Nevertheless, under the protective cover, the fruits can accumulate more soluble solids and vitamin C (Florido and Álvarez, 2015; Shimeles *et al.*, 2017; Bazgaou *et al.*, 2018). In this context, polycarbonate can be taken into consideration as an alternative cover for greenhouses, due to the high light transmittance and UV protection, aside from being a very light but durable material (Kwon *et al.*, 2017). Evaluations of the cover material for protected tomato cultivation in tropical regions are insufficient, and little information is available about material that would allow more favorable cultivation conditions in these regions, to achieve higher yields without affecting the tomato quality.

For high tomato yields under high temperatures, thermos tolerant hybrids must be used. These can ensure high yields of high-quality fruit, even under abiotic stress (Scarano *et al.*, 2020). The identification of tomato genotypes with high commercial and nutritional quality can help producers chose the most suitable cultivar for each cultivation environment under the agroclimatic conditions of the Amazon region in the summer season. This study analyzed the yield performance of tomato hybrids grown in the open field and in environments covered with agricultural film and polycarbonate in the Amazonian summer, by correlating agronomic performance with fruit quality using principal component analysis.

2. Materials and Methods

Plant material, cultivation environments and experimental design

Three tomato hybrids ['BSDS0060' (Blueseeds), 'Trucker' (Nunhems) and 'Thaíse' (Feltrin)] were grown in three growing environments [open field (O); environment covered with agricultural film (F) and polycarbonate panels (P)]. The study was arranged in a randomized block design (CRD) in a factorial arrangement (3x3) with five replications and seven plants each.

The hybrids for the study had an indeterminate growth habit; fruits suited for salad and were chosen because of their yield and disease resistance. 'DS0060' has a late cycle, firm fruits and high fruit cracking resistance; mean fruit weight of 220 to 260g and is tolerant to Tomato spotted wilt virus (TSWV), Tomato mosaic virus (ToMV), Tomato yellow leaf curl virus (TYLCV), *Fusarium oxysporum* f. sp. *lycopersici* (FOL) race 1 and 2 and Verticillium (V) race 1. 'Trucker' is a vigorous F_1 hybrid with excellent leaf cover; mean fruit weight of 240 g and tolerance to TYLCV, TSWV, FOL, V and nematodes. 'Thaise' is a medium-vigor F_1 hybrid with bright red fruits, excellent market standard due to the flavor, fruit uniformity and long shelf life; mean fruit weight of 230 g and tolerance to TYLCV, ToMV, *Verticillium dahliae* Kleb., *Fusarium oxysporum* f. sp. *lycopersici* (FOL) race 3 and root-knot nematode.

The tomato hybrids were grown in the open field and under the protection of a chapel-shaped greenhouse (6.4 x 20 m), lateral height 3.5 m, central height 4.8 m in the north/south direction and side closure with 30% Aluminet, a thermo-reflective shading screen. As cover material of the structure, a low

density transparent agricultural film (F), with UV-A/UV-B protection, 90% transmission, and 25% light diffusion (Nortene 150 µm) was compared with transparent polycarbonate panels (P), with 10 mm thick, a double-layer honeycomb structure and UV-A/UV-B protection (Polisystem).

Area and cultivation conditions of tomato plants

The study was carried out in summer 2019/2020 (November to April) in Sinop, Mato Grosso, Brazil (lat. 11° 52' 12" S, long. 55° 35' 54" W; 364 m asl). According to the Köppen classification, the climate is equatorial savanna with dry winters (Aw), with a mean annual temperature of 25.4°C, a maximum of 34°C, annual rainfall of 1801 mm, and a rainy season between October and April.

The tomato seedlings were produced in a climatized greenhouse, planted in the 162 cells of polystyrene trays, containing 31 ml of commercial substrate (Vivato) per cell. The seedlings were planted 29 days after sowing (DAS) in furrows spaced 1.25 m apart and 0.35 m between plants, with a total population of 22,000 plants per hectare. The plants were trellised by the "Florida weave" method, on a structure of 1.3 m high wooden stalks and twine inserted horizontally every 0.4 m to hold up the plants.

The soil at the site was classified as dystrophic red-yellow latosol (LVA). The chemical properties (0-0.2 m layer) are shown in Table 1. Acidity was corrected with 3.0 t ha^{-1} dolomitic lime (90% total neutralizing power), fertilization at planting consisted of 3.4 t ha^{-1} single superphosphate and 30 t ha^{-1} barnyard manure, incorporated to a depth of 0.2 m with a rotary hoe. Topdressing was applied by drip fertigation, distributed in 10 applications throughout the cycle, containing a total of 120 g calcium nitrate (15% N and 19% Ca), 40 g potassium sulfate (48% K_2O and 15% SO_4), 30 g phosphate monophosphate (12% N and 61% P_2O_5), 110 g potassium nitrate (13% N, 44% K_2O and 1.5% S) and 70 g magnesium sulfate (9% Mg and 12% S) per plant.

Irrigation was applied at a mean net depth of 3.5 mm per day, to compensate for the calculated mean daily evapotranspiration (Valeriano *et al.*, 2017). Diseases and pests were controlled as recommended for the crop, by monitoring and applying products (based on pyraclostrobin, fluxapyroxad, trifloxystrobin, prothioconazole, kasugamycin, copper oxychloride, equivalent in metallic copper, mancozeb, carbosulfan, abamectin, haloxyfop-p-methyl, pyriproxyfen, acetamiprid, alpha-cypermethrin, chlorfenapyr, beauveria bassiana) in active principle rotation and at rates recommended by the manufacturer. Weed was controlled by hand weeding between plants and in-between rows.

Table 1 - Soil physicochemical analysis in the experimental area

Physico-chemical characteristics	Data
pH water	5.1
pH $CaCl_2$	4.3
P (mg $dm^{-3)}$	0.9
K (mg dm^{-3})	37
Ca + Mg	1.0
Ca ($cmol_c$ dm^{-3})	0.8
Mg ($cmol_c$ dm^{-3})	0.2
Al ($cmol_c$ dm^{-3})	0.5
H ($cmol_c$ dm^{-3})	3.9
OM (g dm^{-3})	20.0
Sand (g Kg^{-1})	283
Silt (g Kg^{-1})	133
Clay (g Kg^{-1})	584
Sum of bases	1.1
CEC	5.4
V (%)	20.1
Ca/Mg ratio	3.2
Ca/K ratio	8.3
Mg/K ratio	2.6
Ca Sat.	14.7
Mg Sat.	4.6
Al Sat.	30.2
K Sat.	1.8
H Sat.	71.2

Monitoring environmental variables

The microclimatic variables (temperature, relative humidity, global radiation and PAR) of each environment were monitored and recorded at meteorological stations (U30, HOBO) equipped with Sigma sensors installed at the center of each environment, at a mean height of 1.80 m. Readings were taken every 20 min and data compiled in hourly mean per month from 6:00 am to 6:00 pm. Rainfall data were collected at the station installed in the open-field environment from December 12, 2019 to April 17, 2020.

Assessment of agronomic characteristics

Ripe fruits were harvested at ripening stage 6 (intense red color on more than 90% of the fruit surface) (Skolik *et al.*, 2019). Fruits were harvested somewhere between 99 and 137 d.a.s. within a peri-

od of about nine days in the protected environments. In the open field, harvest was already carried out 99 d.a.s. due to the poor phytosanitary state of the crop. The total fruit weight (kg plant^{-1}) and total number of fruits were immediately determined and then the commercial standard of the fruits was classified to (i.e., appearance, size and damage level) to determine the commercial fruit weight and number of commercial fruits, underlying the estimation of the overall yield (t ha^{-1}) of 22 thousand plants ha^{-1}.

Evaluation of photosynthetic responses

Photosynthetic parameters were evaluated with a portable infrared photosynthesis analyzer (LCi-SD, ADC). Photosynthetically active radiation (PAR, µmol m^{-2} s^{-1}), net CO_2 assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), leaf transpiration rate (E, mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration in the substomatal chamber (C_i, µmol mol^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}). The fourth fully expanded leaf from the plant apex during the harvest period (i.e., fruit filling; between 128 and 136 d.a.s) was used. The measurements were carried out on a sunny and cloudless day between 8 and 10 am. Readings were taken in all treatments except on the open field, due to the high degree of crop damage caused by diseases.

Preparation of tomato samples

Fruits of the nine treatments harvested at 99 DAS were selected according to the market standards and samples of 12 fruits per plot were separated. The material was sanitized by immersion in chlorinated water (100 mg L^{-1} sodium hypochlorite) for 10 min and washed in distilled water. For physicochemical and biochemical analyses, the tomatoes were blended in a food processor (Philips Walita®). All samples were stored in triplicate at -80°C for later analysis.

Physicochemical and biochemical analyses

Fruit flesh firmness, total soluble solids, titratable acidity and ripening index

Flesh firmness of the tomatoes was measured with a penetrometer (TA HD Plus, Stable Micro System) by inserting a 6 mm tip into the skinless fruits to a depth of 9 mm. Soluble solids were determined in a refractometer (PAL-BX/RI, Atago). Titratable acidity (TA) was determined by the procedure described by Zenebon *et al*. (2005), using a benchtop pH meter (Hanna Instruments HI901). Results were expressed in % of citric acid, calculated by the formula:

$$TA = \frac{V \times fc \times 10}{P} \times 100 \quad (1)$$

where V is the volume (in mL) of 0.1 M NaOH used for titration; fc is the correction factor of NaOH and P the sample weight (in g). The fruit ripening index (ratio) was calculated as the ratio between total soluble solids and titratable acidity.

Fruit color

The color coordinates were read with a colorimeter (Color Quest XE, Hunter Lab). Readings were performed in the L*a*b system. The chromaticity (C*) and Hue angle were determined by the formulas:

$$C = (a + b)^{½} \quad (2)$$

$$Hue = tg^{-1}(b/a) \quad (3)$$

Lycopene and β-carotene contents

The lycopene and β-carotene contents were determined as proposed by Nagata and Yamashita (1992). A 1-g sample was homogenized in 10 ml acetone:hexane (4:6 v/v) solution in a turrax blender. The resulting solution was analyzed in a spectrophotometer (Evolution 201, Thermo Scientific) after phase separation. Absorbance was determined at 453, 505, 645 and 663 nm and the results (mg 100^{-1} g) computed by the formulas:

$$Lycopene = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (4)$$

$$ß\text{ - }carotene = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (5)$$

Data analysis

The data were subjected to analysis of variance (ANOVA) and the means compared by the Scott-Knott test (P<0.05), using software SISVAR version 5.6 (Ferreira, 2019). Principal component analysis (PCA) was run on software XLSTAT version 2021.3.1.

3. Results and Discussion

Agroclimatic variables

Data on rainfall and daily variations in global radiation, PAR, temperature and relative humidity in the cultivation environments during the experimental period were recorded (Fig. 1A-1E). The conditions of high rainfall and high temperatures restricted the plant cycle to 99 d.a.s. The total rainfall volume was 1841.9 mm, of which 633.1 mm fell between flowering and fruit formation and 531.6 mm during fruit filling and harvesting (Fig. 1A). The air temperature varied from 22 to 29°C (Fig. 1D). For tomato cultivation,

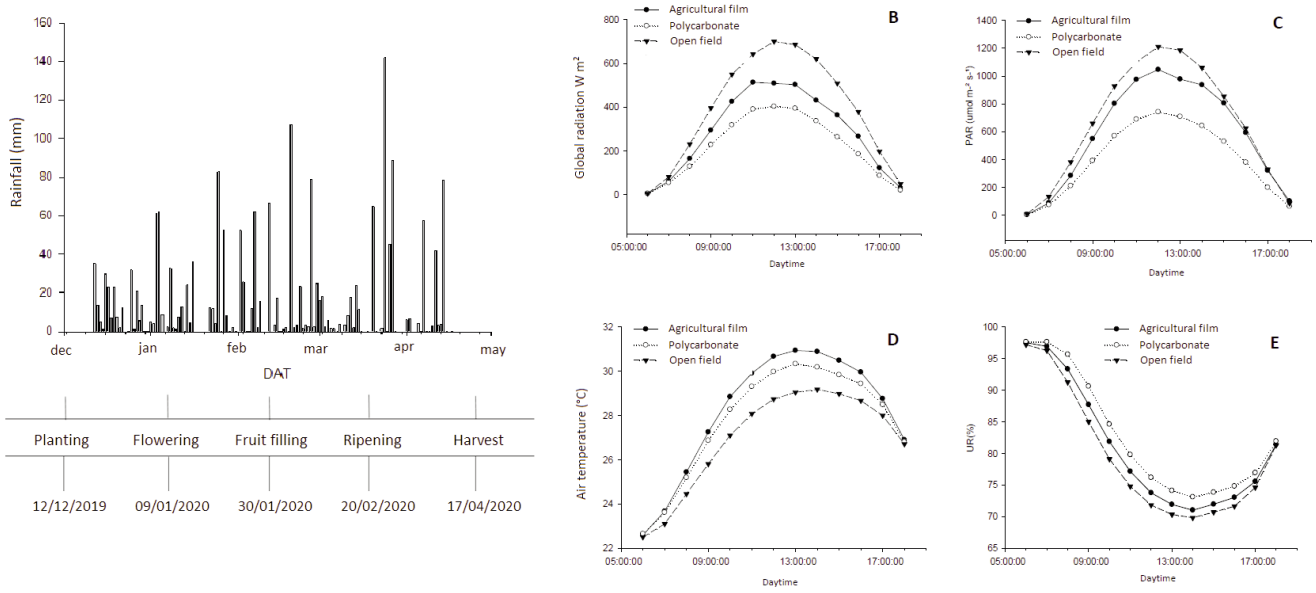

Fig. 1 - Rainfall during the experimental period (A) and daily variation of global radiation (B), PAR (C) air temperature (D) and relative humidity (E) in different growing environments.

the optimal air temperature range is 20 to 24°C during the day and 18°C at night (Shimeles *et al*., 2017). Above 29°C, yields decrease due to reduced fruit set (Harel *et al*., 2014). Thus, the high rainfall volume and high temperatures limited open-field tomato cultivation by favoring high disease severity, which impaired the photosynthetic analysis in this plant group. Protected cultivation provided an efficient barrier against excessive rainfall, but raised the maximum temperatures by 6.3% in environment F and by 4.4% in P, compared to the open field. The relative air humidity was quite similar in the evaluated cultivation environments.

Regarding luminosity, global radiation and PAR were lower in environments P and F than in the open field (Figs. 1B and 1C). In environment P, global radiation reached 403.3 W and PAR 742.6 µmol m^{-2} s^{-1} at noon, i.e., about 40% less than in the open field and 25% lower than in environment F. Under low light incidence, tomato has optimized efficiency of PAR use (Radin *et al*., 2003). This information was confirmed in our study by the higher tomato yield of plants grown in environment P (Table 2). The analysis of the photosynthetic responses (Table 3), net CO_2 assimilation rate (*A*), leaf transpiration rate (E), internal CO_2 concentration (*Ci*) and stomatal conductance (*Gs*) detected no differences between the protected environments. In environment P, the *A* value of hybrid 'Trucker' was 118% higher than in F, with a yield increase of 52% (Table 2). This variation can be attributed to the genetic characteristics of the hybrids and their responses to agroclimatic conditions (i.e., global radiation, PAR, air temperature). In a study of Kwon *et al*. (2017), the *A* of hybrid 'Superdoterang' grown in environments under polycarbonate and glass, respectively, did not differ (24.8 and 21.8 µmol m^{-2} s^{-1}). In this study, the better response of cultivars to the conditions in environment P may be related to a better light capture (of diffuse radiation) at a more efficient wavelength for photosynthesis, ass similarly observed elsewhere (Radin *et al.*, 2003; Kwon *et al*., 2017).

Yield of tomato hybrids in different growing environments

The protected environments provided significantly higher fruit yields. The marketable fruit weight of the hybrids increased 7-fold in environment P and 4.5-fold in F, compared to the open field (Table 2). These results were better than those of Yeshiwas *et al.* (2016), who reported a 54% increase in tomato yield in a protected environment compared to an open field. 'Thaise' performed well under both cover types (3.90 and 3.27 kg $plant^{-1}$), while 'DS0060' and 'Trucker' had higher yields in environment P (3.23 and 3.57 kg $plant^{-1}$, respectively). The thermotolerance of 'Thaise' was better, making the hybrid adaptable to a wide temperature range, which resulted in

Table 2 - Total fruit weight per plant, weight of marketable fruits, total number of fruits, number of marketable fruits and total yield of tomato hybrids grown in the open field (O) and protected environments covered with agricultural film (F) and polycarbonate (P)

	Hybrid (H)			Mean	F (ANOVA)			CV%
	DS0060	Trucker	Thaíse		A	H	E x H	
Total fruit weight (kg/plant)								
O	0.384 cA [z]	0.860 bA	1.192 bA	0.812 c				
F	2.214 bB	2.636 aB	3.924 aA	2.924 b	54.0 **	5.91**	0.50 NS	12.7 [y]
P	3.424 aA	4.006 aA	4.208 aA	3.879 a				
Mean	2.007 B	2.500 B	3.108 A					
Weight of marketable fruits (kg/plant)								
O	0.192 cA	0.576 cA	0.572 bA	0.446 c				
F	1.834 bB	2.256 bB	3.276 aA	2.455 b	66.4 **	3.60 *	0.55 NS	13.1 [y]
P	3.236 aA	3.576 aA	3.900 aA	3.570 a				
Mean	1.754 A	2.136 A	2.582 A					
Total number of fruit (fruits/plant)								
O	3.60 bB	8.60 bA	12.4 bA	8.20 b				
F	21.0 aB	27.8 aB	48.2 aA	32.3 a	52.2 **	14.0 **	0.86 NS	17.9 [y]
P	24.8 aA	36.6 aA	39.4 aA	33.6 a				
Mean	16.4 C	24.3 B	33.3 A					
Number of marketable fruits								
O	0.80 bA	4.40 bA	3.20 bA	2.80 b				
F	15.2 aB	20.2 aB	35.0 aA	23.4 a	86.2 **	9.73 **	1.51 NS	19.5 [y]
P	20.2 aB	30.0 aA	33.4 aA	27.8 a				
Mean	12.1 B	18.2 A	23.8 A					
Total yield (t ha^{-1})								
O	8.44 cB	18.9 bA	26.2 bA	17.8 c				
F	48.7 bB	58.0 aB	86.2 aA	64.3 b	64.0 **	7.08 **	0.61 NS	17.7 [y]
P	75.3 aA	88.2 aA	92.5 aA	85.3 a				
Mean	44.1 B	55.1 B	68.3 A					

[z] Means followed by the same uppercase letter in the rows or lowercase letter in the columns do not differ statistically from each other by the Scott-Knott test at 5%.
[y] Data transformed into $\sqrt{y+1}$.
** P<0.01, * P<0.05, NS= Not significant P>0.05.

the best performance in environment F, with a total fruit weight of 3.92 kg plant^{-1} and marketable fruit weight of 3.27 kg plant^{-1}, which can be considered reasonable for tomato cultivation at high temperatures (Scarano *et al.*, 2020). The yield recorded in this study exceeded that of 'Superdoterang' which produced 2.8 kg of fruit plant^{-1} in a protected environment covered with polycarbonate (Kwon *et al.*, 2017) and of 'Bishola', with determinate growth habit, which produced 1.81 kg per plant (Yeshiwas *et al.*, 2016).

The total number of fruits was higher in 'Thaíse' in both environments, F and P, while the results of 'Trucker' were better in P (Table 2). However, a higher percentage of fruit of 'Thaise' had to be discarded in environment F (27%) than in environment P (15%). This was most likely caused by the higher global radiation, PAR and air temperature in environment F (Fig. 1B, 1C and 1D).

Tomato cultivation in the Amazon region in the summer is high risk farming, due to the occurrence of rain causing waterlogging of the soil and leaf wetting, which are rather unfavorable factors, particularly when associated with heat. In tropical regions, depending on the year of cultivation and plant management, severe disease and pest damage can occur at the harvest stage (Subin *et al.*, 2020). In this study, fruit loss in the open field was high, causing a decrease of 78% in the number of fruits in 'DS0060', 49% in 'Trucker' and 75% in 'Thaise'. This resulted from the unfavorable agroclimatic conditions during the growing season. High rainfalls together with heat

Table 3 - CO_2 net assimilation rate (A), leaf transpiration rate (E), internal CO_2 concentration in the substomatal chamber (Ci) and stomatal conductance (Gs) in tomato hybrids grown in open field (O), environments protected covered with agricultural film (F) and polycarbonate (P)

Photosynthetic variable	Hybrid (H)			Mean	F (ANOVA)			CV%
	DS0060	Trucker	Thaíse		E	H	E x H	
A (µmol CO_2 m^{-2} s^{-1})								
O	-	-	-	-				
F	11.0 aA [z]	9.24 bA	12.9 aA	11.1 a	2.09 NS	3.51 NS	5.67 *	14.4 [y]
P	7.67 aB	20.1 aA	14.9 aA	14.2 a				
Mean	9.35 B	14.6 A	13.9 A					
E (mmol H_2O m^{-2} s^{-1})								
O	-	-	-	-				
F	3.68 aA	5.45 aA	4.96 aA	4.70 a	0.10 NS	3.24 NS	0.11 NS	12.9
P	3.07 aA	5.36 aA	4.86 aA	4.43 a				
Mean	3.37 A	5.41 A	4.91 A					
Ci (µmol mol^{-1})								
O	-	-	-	-				
F	349.3 aA	373.7 aA	315.0 aA	346.0 a	3.79 NS	0.28 NS	1.05 NS	6.77
P	306.7 aA	295.0 aA	312.3 aA	304.6 a				
Mean	328.0 A	334.3 A	313.7 A					
Gs (mol m^{-2} s^{-1})								
O	-	-	-	-				
F	0.326 aA	0.336 bA	0.690 aA	0.451 a	1.74 NS	2.46 NS	1.57 NS	11.4
P	0.290 aA	1.001 aA	0.790 aA	0.696 a				
Mean	0.3 A	0.6 A	0.7 A					

[z] Means followed by the same uppercase letter in the rows or lowercase letter in the columns do not differ statistically from each other by the Scott-Knott test at 5%.
[y] Data transformed into $\sqrt{y+1}$.
** P<0.01, * P<0.05, NS= Not significant P>0.05.

are factors that increase the risk of disease incidence in tomato crops (Silva *et al*., 2013). In tropical regions, high pest and pathogen pressure jeopardize production. In this study, 46% of the plants were decimated, and between flowering and the beginning of harvest, the disease severity index had reached a maximum level in all cultivated areas, minimizing the plant yield.

When estimating the total yield, 'Thaíse' cultivated in environment F or P (86.2 and 92.5 t ha^{-1}, respectively) together with 'DS0060' and 'Trucker' cultivated in environment F (75.3 and 88.2 t ha^{-1}, respectively) were the highest yielding (Table 2). These yields are considered high when compared to the hybrids 'Lampião' (73.0 t ha^{-1}), 'Fascínio' (68.3 t ha^{-1}), 'Candieiro' (66.1 t ha^{-1}) and 'Shanty' (62. 4 t ha^{-1}) produced under similar growing conditions as in this study (i.e., protected environment, tropical climate, high temperatures)(Seabra *et al.*, 2022). These results confirm the high yield potential of the evaluated tomato hybrids in the interim growing season, under protected cultivation and at high temperatures. The high yields recorded must be related to the thermotolerance of the evaluated genetic material, mainly of 'Trucker' and 'Thaise', resulting in higher profits for producers.

Physicochemical and biochemical properties of fruits in response to growing environments

Tomato color is an important quality property. The fruits grown in the open field had higher values of luminosity (L*), hue angle (h°) and higher b* coordinates, indicating lighter, brighter and more yellowish fruits. On the other hand, the fruits produced in environments F and P had higher a* coordinates, with more reddish fruits (Table 4). The fruits of hybrid 'DS0060' had higher chromaticity (C*), L*, h° and b* coordinate values, mainly when grown in the open field, also indicating lighter colored fruits, while the a* coordinates of 'Trucker' and 'Thaise' were higher, i.e., the reddish fruit color was more intense. These results showed that the adaptation of hybrid

Table 4 - Chromaticity (C*), luminosity (L*), hue angle (h°), coordinates (a* and b*) of fruits of tomato hybrids grown in open field (O) and protected environments covered with agricultural film (F) or with polycarbonate (P)

Variable	Hybrid (H)			Mean	F (ANOVA)			CV%
	DS0060	Trucker	Thaíse		A	H	A x H	
*C**								
O	51.1 aA *z*	50.8 aA	51.1 aA	51.0 a				
F	52.0 aA	49.8 aB	49.3 aB	50.3 a	1.81 NS	4.10 *	0.90 NS	7.59
P	51.1 aA	49.6 aA	48.8 aA	49.8 a				
Mean	51.4 A	50.0 B	49.7 B					
*L**								
O	51.9 aA	49.4 aB	45.0 aC	48.8 a				
F	46.0 bA	42.8 bB	42.3 bB	43.7 b	35.9 **	28.8 **	2.06 NS	8.66
P	46.7 bA	43.9 bB	42.7 bB	44.4 b				
Mean	48.2 A	45.4 B	43.3 C					
h°								
O	60.7 aA	53.7 aB	44.7 aC	53.0 a				
F	44.8 bA	40.6 bB	39.7 bB	41.7 b	58.9 **	31.6 **	5.27 **	14.8
P	46.5 bA	43.4 bB	40.8 bB	43.6 b				
Mean	50.7 A	45.9 B	41.8 C					
*a**								
O	24.9 bC	29.9 bB	36.2 aA	30.3 b				
F	36.4 aA	37.4 aA	37.8 aA	37.2 a	40.1 **	18.2 **	7.74 **	14.5
P	34.8 aA	35.7 aA	37.0 aA	35.8 a				
Mean	32.0 C	34.3 B	37.0 A					
*b**								
O	43.8 aA	40.3 aB	35.9 aC	40.0 a				
F	36.4 bA	32.6 bB	31.4 bB	33.5 b	38.5 **	25.9 **	1.00 NS	13.9
P	36.7 bA	34.0 bB	31.9 bB	34.2 b				
Mean	39.0 A	35.7 B	33.1 C					

(z) Means followed by the same uppercase letter in the rows or lowercase letter in the columns do not differ statistically from each other by the Scott-Knott test at 5%.
** P<0.01, * P<0.05, NS Not significant P>0.05.

'DS0060' to high solar radiation and heat was poor, and it should be evaluated in growing seasons with milder temperatures.

In this study, the fruits were harvested when more than 90% of the fruit surface had become deeply red (stage 6). The fruit flesh of the tomatoes from the open field was firmer (Table 5). Of the hybrids, 'Trucker' had the firmest fruit flesh. This characteristic is relevant with regard to transport resistance, and is influenced by the ripening stage of the fruit, and possibly by the genetic and environmental characteristics of cultivation.

In general, the levels of soluble solids were slightly higher in tomatoes from environment F (Table 5). Among the hybrids, 'DS0060' had the highest soluble solids content. These results are similar to those published by Kwon *et al.* (2017) who found contents between 5.1 and 5.2°Bx, but observed no difference for this variable between cultivation environments. According to the authors, soluble solids may be strongly genetically influenced. 'Lampião' tomatoes, which are sweeter, contained 4.12°Bx and 'Fascínio' 3.48°Bx. According to the above authors, consumers prefer tomatoes with 4.0 to 6.0°Bx (Domiciano *et al*., 2021). In this study, all hybrids produced fruits with soluble solids contents above 4°Bx. Soluble solids in tomato consist mainly of reducing sugars. Thus, agronomic factors (i.e., seasonal climate variation, management practices) that alter the photosynthetic activity, and consequently sucrose synthesis, can modify glucose and fructose accumulation in fruits, and thus the soluble solids contents (Yeshiwas *et al.,* 2016).

The titratable acidity of the fruits ranged from

Table 5 - Fruit flesh firmness, soluble solids, tritable acidity, ripening index, lycopene and β-carotene contents in fruits of tomato hybrids grown in open field (O) and protected environments covered with agricultural film (F) or with polycarbonate (P)

Variable	Hybrid (H)			Mean	F (ANOVA)			CV%
	DS0060	Trucker	Thaíse		A	H	AXH	
Fruit flesh firmness (N)								
O	9.16 aB [z]	12.4 aA	9.64 aB	10.4 a				
F	7.00 bB	8.32 bA	6.08 bB	7.13 b	30.0 **	12.1 **	1.10 ns	37.9
P	7.00 bA	7.92 bA	5.84 bA	9.62 b				
Mean	7.72 B	9.56 A	7.18 B					
Soluble solids (°Bx)								
O	4.46 bA	4.00 bB	4.06 bB	4.17 c				
F	5.20 aA	4.66 aB	4.60 aB	4.82 a	20.5 **	11.2 **	0.90 ns	10.7
P	4.60 bA	4.46 aA	4.26 bA	4.44 b				
Mean	4.75 A	4.37 B	4.31 B					
Tritable acidity (%)								
O	0.340 aA	0.300 aB	0.280 bB	0.306 a				
F	0.300 bB	0.273 bB	0.333 aA	0.302 a	1.32 ns	4.88 **	8.6 **	12.8
P	0.300 bB	0.306 aB	0.340 aA	0.315 a				
Mean	0.313 A	0.293 B	0.317 A					
Ripening index (SS/TA)								
O	13.1 cA	13.3 bA	14.5 aA	13.6 b				
F	17.3 aA	16.5 aA	12.9 bB	15.6 a	27.4*	12.1*	17.8*	12.3
P	15.3 bA	15.5 aA	13.5 aA	14.8 a				
Mean	15.2 A	15.1 A	13.6 B					
Lycopene [z] (mg 100 g^{-1})								
O	0.395 bB	0.581 cA	0.669 cA	0.548 c				
F	0.861 aC	1.127 aB	1.467 aA	1.150 a	114.7 **	33.9 **	5.36 **	11.3 [y]
P	0.751 aA	0.782 bA	0.833 bA	0.788 b				
Mean	0.669 C	0.828 B	0.989 A					
β-carotene (mg 100 g^{-1})								
O	0.378 bB	0.509 cA	0.574 cA	0.487 c				
F	0.727 aC	0.953 aB	1.231 aA	0.907 a	105.0 **	35.0 **	3.91 **	10.8 [y]
P	0.639 aB	0.681 bB	0.769 bA	0.695 b				
Mean	0.581 C	0.714 B	0.856 A					

[z] Means followed by the same uppercase letter in the rows or lowercase letter in the columns do not differ statistically from each other by the Scott-Knott test at 5%.
[y] Data transformed into $\sqrt{y+1}$.
** P<0.01, * P < 0.05, NS Not significant P > 0.05.

0.27 to 0.34%. There was a significant interaction between environments and hybrids, resulting in the highest acidity in fruits of 'DS0060', grown in the open field, and of 'Thaise' produced in environment F or P (Table 5). These values were lower than the 0.39 to 0.55% reported by Scarano *et al.* (2020), but similar to the range of 0.22 to 0.32% found by Nour *et al*. (2015). Acidity is influenced by the moment of fruit harvest and possibly also by genetic characteristics of the hybrids. The sweetness acidity ratio or relationship between sweetness and acidity, called ripening index, determines the taste, indicating a mild or acid flavor. Fruits with an index equal to or greater than 10 are considered ideal for consumption (Kader and Stevens, 1978). The index in this study exceeded 13, reaching 17.3 in 'DS0060' fruits from environment F (Table 5). For 'DS0060' and 'Trucker', the ripening index in open field cultivation was lower. However, due to the high soluble solids level, all environments and hybrids produced fruits with good market acceptance, i.e., a high ripening index, mainly due to the determined moment of harvest, when more than

90% of the fruit surface had become deeply red, ideal for marketing of the product in the region.

Regarding the carotenoid content of the fruits, the lycopene and β-carotene contents were higher in environment F and lower in open-field tomato (Table 5). This result can be explained by the high incidence of solar radiation on the plants (Fig. 1B). The contents were superior to those reported for *saladette* tomato produced in a protected environment at high temperatures, ranging from 0.3 to 0.82 mg 100 g^{-1} for lycopene and 0.06 to 0.09 mg 100 g^{-1} for β-carotene (Domiciano *et al*., 2021). Among the hybrids, 'Thaise' contained the highest and 'DS0060' lowest levels of these two compounds. The different lycopene contents in the hybrids can be attributed to genetic characteristics, climate, location, cultivation method and fruit ripening stage. According to Nour *et al.* (2015), carotenoid synthesis, mainly of lycopene, is influenced by the genetic characteristics of adaptability to the agroclimatic conditions of the cultivation environment.

Significant positive correlations were observed between the a* coordinate (Table 4) and lycopene (r = 0.743) and β-carotene (r = 0.742) levels. On the other hand, correlations were negative between the content of these carotenoids and L* (r = -0.793, r = -0.810), h° (r = -0.789, r = -0.798) and b* coordinate (r = -0.818, r = -0.837). These results are similar to those reported by Nour *et al.* (2015), who found a negative correlation of the L* value with the lycopene content in tomato. In this study, open-field fruits of 'DS0060' also had mean L* and lower lycopene and β-carotene levels (Tables 4 and 5). For 'Belladona', the chromaticity values were related to the carotenoid contents, except for lycopene (Papaioannou *et al*., 2012). However, this information was not confirmed by the results of this study, where the chromaticity values were weakly correlated with lycopene (r = -0.526) and β-carotene (r = -0.579) contents.

Considering all study variables, PCA analysis grouped 'Thaise' grown in environments F and P and 'Trucker' grown in environment P on the PC1+, corresponding to 65.84% of the data, with the best results in terms of yield as well as quality characteristics (Fig. 2). Hybrids 'DS0060', 'Trucker' and 'Thaise' cultivated in the open field (PC1-) produced low yields, fruits with undesirable color and very firm fruit flesh due to the environmental conditions that stressed the plants, causing high yield losses and physiological disorders. The hybrids 'DS0060' and 'Trucker' cultivated

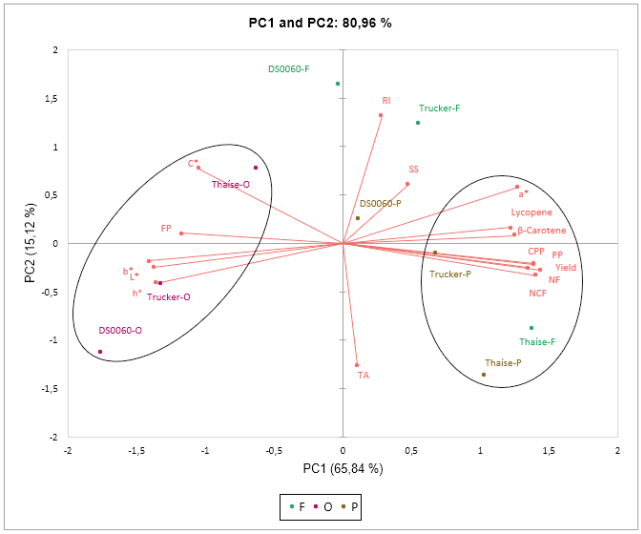

Fig. 2 - Two-dimensional projection and score of productive characteristics (yield; plant production—PP commercial plant production—CPP; number of fruits – NF, number of commercial fruits – NCF, chroma - C*, luminosity - L*, hue angle - h°, coordinates - a* and b*, fruit firmness – FP, soluble solids - SS, titratable acidity – TA, ripening index - RI, lycopene and β-carotene) of tomato cultivars (DS0060, Trucker, and Thaíse) in response to different environments – open field (O), agricultural film (F), and polycarbonate.

in environment F were grouped in PC2+, with low acidity and high fruit ripening indices, probably due to low heat tolerance. These results reinforce the relevance of cultivation in protected environments to warrant high tomato yield during the hot and humid summers of the Amazon region. Equally important is the selection of hybrids adapted to high radiation and high temperatures, which are fairly common under these cultivation conditions.

Protected environments were indispensable to achieve high yields under the unfavorable agroclimatic conditions (i.e., high rainfall, high solar radiation, high temperatures) of the southern Amazon region in the interim crop season (January to April). The protected environment covered with polycarbonate panels considerably increased the fruit yield of the tomato hybrids evaluated, indicating it as a good alternative for tomato cultivation in tropical regions. In protected environments covered with agricultural film or polycarbonate panels, hybrid 'Thaise' produced high yields with good quality characteristics. 'Trucker' stood out with the firmest fruit flesh and lowest acidity. However, 'Thaise' had the highest lycopene and β-carotene levels, a character-

istic that appeals to more demanding consumers, since these compounds are strongly related to disease prevention, and their presence in the human diet is essential.

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Shelf-life and post-harvest quality of tomato (*Lycopersicon esculentum* Mill.) varieties to different packaging materials at Mersa, North Wollo, Ethiopia

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Authors contributions:
The authors (Dr. Seid Hussen and Dr. Biruk Masrie) developed the idea and initiation, edit the draft the proposal, and write the manuscript. The co-author (Solomon Worku) conducted the research, did data collection and analysis.

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Abstract: Tomato *(Lycopersicon esculentum Mill.)* has a short shelf life at ambient conditions and is a highly perishable crop. Extreme post-harvest losses occur as a result of the wrong packaging materials. However, by employing the right packing materials, tomato varietals can have longer shelf lives. Globally rising fresh tomato demand has forced the development of essential mechanisms, including packaging materials, to improve shelf life. The current study was initiated to evaluate the quality and shelf-life of tomato varieties in response to packaging materials at Mersa, North Wollo, Ethiopia, during 2021 cropping season. Three replications of a completely randomized design were used to test three tomato varieties (Roma VF, Oval red and Woyno) and seven packing materials [closed carton (CC), open carton (OC), closed wooden box (CWB), open wooden box (OWB), perforated polyethylene bag (PPB), non-perforated polyethylene bag (NPPB) and control (C)] at room temperature (20-22°C). According to the findings, there is a significant (P<0.05) interaction effect between packaging materials and varieties on a number of parameters, including physiological weight loss, decay percentage, disease incidence, total soluble solids (TSS), tomato fruit PH, juice color score, overall acceptability, marketability percentage, and shelf life. Non-perforated polyethylene plastic experienced the highest physiological weight losses of 79.88% and 79.63% after 18 days of storage. Roma VF variety showed the greatest weight loss. In addition, PPB showed the lowest decay percentage (20%) and maximum marketability (20%) during the 18th day of storage. At the end of storage, NPPB with Roma VF and Woyno varieties had a substantially (100%) larger decay loss of tomato fruits. NPPB has been linked to the highest disease incidence (20%). Roma VF and Oval red recorded the highest pH tomato fruit's color and overall acceptability score on PPB. It can, thus, be concluded that packaging of tomato fruits in PPB can extend shelf-life with better-quality of the produce. However, to develop plausible recommendation, the study should be repeated in multi-location with more packaging methods and varieties over seasons.

1. Introduction

The tomato (*Lycopersicon esculentum* Mill.) crop, which was domesticated in Mexico, is the most commonly grown vegetable crop in the world. From the equator to Chile, the western coastal plain of South America is where it originated (Mapes and Basurto, 2016). It ranks first on the list of canned vegetables and is the most extensively consumed vegetable crop, followed by the potato and sweet potato (Yesdhanulla and Aparna, 2018). The cultivated tomato was first used in Ethiopian agriculture between 1935 and 1940. It is one of the most important crops grown by smallholder farmers in Ethiopia. Tomatoes are essentially a perennial plant, even though they are farmed as an annual crop. They are regarded as a delicate warm-season crop that is prone to cold (Abdu, 2016).

Tomatoes have a relatively short shelf life due to several postharvest physiological, physical, and chemical changes that occur during storage (Haile and Safawo, 2018). Various methods and strategies are being evaluated to minimize postharvest losses and enhance their storage life. Because they are a climacteric and perishable fruit, tomatoes have a very short shelf life, under normal circumstances (Caroline *et al.*, 2015; Ayomide *et al.*, 2019). Fruit rot, inappropriate handling and storage techniques, and external injury received during harvest are the main causes of post-harvest losses. A tomato fruit's fresh weight is 90% water, and the size of the fruit is influenced by the water supply to the plant. Fruit with this much water content is perishable. The majority of fruits and vegetables suffer from water loss during storage, which is impacted by temperature (>55°F) and relative humidity (<80%) conditions (Bonazzi and Dumoulin, 2011).

Despite the growth of Ethiopia's horticultural sector, there is still a lack of funds to address post-harvest loss and crop quality issues (Kasso and Bekele, 2018). Post-harvest losses of horticulture crops in Ethiopia were observed to range from 15% to 70% (Urge *et al.*, 2014). Post-harvest loss of horticulture products in Ethiopia was attributed to a number of factors, including transportation, a lack of proper storage facilities, and unsuitable packaging materials (Hagos, 2014; Kasso and Bekele, 2018). Losses during and after harvest are a significant source of food loss because they have a direct impact on people's livelihoods and the whole economy, which is important for food security, nutrition, and lowering poverty.

Prior to marketing, transit, and storage, horticultural crops experience the worst post-harvest loss and quality deterioration. Common horticultural goods' post-harvest loss was attributed primarily to inadequate packaging, poor transportation, inadequate storage, and unfavorable market conditions (Seid *et al.*, 2013). Various studies have explored the magnitude of vegetable postharvest losses, production limitations, and agronomic practices. However, there is a dearth of data on the impact of storage conditions and packing materials on tomato fruit shelf life. Furthermore, the knowledge of different packaging materials and storage methods used by small-holder farmers and customers in Mersa and neighboring districts of Northeastern Ethiopia where the current research was conducted is scarce, despite few experiences across various regions of the country. Tomato growers, distributors and consumers can benefit from choosing the best possible packaging material to preserve tomato quality during harvest and extend shelf life. The objective of this study is to examine the impact of various packaging materials on the postharvest quality and shelf-life of tomato varieties at Mersa, North Wollo, Ethiopia.

2. Materials and Methods

Description of the study area

The current experiment was conducted at Mersa College of Agriculture, Woldia University, North Wollo, Ethiopia. The area is geographically locate at 390° 38' E and 11°35'N with an altitude of 1600 m asl in the semi-arid tropical belt of north-eastern Ethiopia. It is 491 km away from Addis Ababa to the northeast and 30km away from Woldia town to the south. It receives an average annual rain fall ranging from 750 to 1000 mm with a bimodal pattern, short rainy season from February to April and long rainy season from July to September. The average annual temperature is about 28.5°C. The soil texture is clay loam and classified as vertisol. The pH of the soil is slightly acidic to slightly alkaline.

Treatments and experimental design

There were seven packaging materials available; namely open carton (OC), closed carton (CC), perforated polyethylene bag (PPB), non-perforated polyethylene bag (NPPB), open wooden box (OWB), closed wooden box (CWB), and control (without packaging) (C); along with three varieties of tomato;

Woyno, Oval red, and Roma VF. The experiment comprised of 21 treatments with three replications arranged in completely randomized design (CRD). The three tomato varieties were produced independently in a field. Half a Kg of tomato fruits for each replication was used for the experiment. In accordance with specifications of the design, each treatment was assigned randomly to the experimental units within a replication.

Description of experimental materials

Varieties. Three tomato varieties (Roma VF, Woyno and Oval red) were used in the experiment. Seeds of all varieties were obtained from Sirinka Agricultural Research Center located at 20 kms to the north of the experimental site. Their descriptions are indicated in Table 1. Tomato fruits of each variety at the time of harvesting are as shown in the figure below (Fig. 1).

Fig. 1 - Tomato varieties used in the experiment (A=Roma VF, B=Oval red, C=Woyno).

Packaging materials. The treatment consisted of seven different packaging materials (open carton, closed carton, open wooden box, closed wooden box, perforated polyethylene bag, non-perforated polyethylene bag and ambient (without packaging) as control.

Closed carton (CC). A carton made from paperboard with the size of 42 cm length25 cm height and 35 cm width was used. The carton was closed after putting the fruits inside the carton.

Open carton (OC): A carton made from paperboard with the size of 42 cm length, 25 cm height and 35 cm width was used. Fruits were placed inside the carton and were left open.

Closed wooden box (CWB). A wooden box made from wood with the size 40 cm length, 30 cm height and 30 cm width was used. The box was closed after putting the fruits inside it.

Open wooden box (OWB). A wooden box made from wood with the size 40 cm length, 30 cm height and 30 cm width was used. Fruits were placed inside the wooden box and were left open.

Perforated polyethylene bag (PPB). A Perforated polyethylene bag made from plastic with 0.4mm thickness of white polyethylene bag and it was having 25% hole designed for the experimentation purpose, were obtained from market. Fruits were placed in perforated polyethylene bag.

Non-perforated polyethylene bag (NPPB). A non-Perforated polyethylene bag made from plastic with 0.4 mm thickness of white polyethylene bag designed for the experimentation purpose, were obtained from market. Fruits were placed in non-perforated polyethylene bag.

Control (without packaging) (C). Fruits were placed on open table at room temperature in laboratory without any packaging**,** were designed for the experimentation purpose.

Experimental management

Seeds were sown in rows of 15 cm spacing on well prepared raised nursery beds having the size of 1 m x 1 m (for each variety) at Mersa Habru Agricultural and Rural Development Office fruit nursery site. Seeds were covered lightly with fine soil and with

Table 1 - Description of tomato varieties used for the experiment

Description	Types of Varieties		
	Roma VF	Woyno	Oval red
Year of release	1977	2006	2007
Altitude (masl)	700-1900	800-2000	800-2000
Growth habit	Determinate	Determinate	Determinate
Fruit shape	Globular	Oblong	Oval
Utilization	Fresh	Fresh	Fresh
Maturity (days)	95-100	100-120	100-110
Yield Research field	400	45	42
Yield Farmer`s field	120-140	13-17	14-18

two-three cm thick grass mulch. Transplanting of seedlings to experimental field was done when seedlings attain the height of about 13-15 cm and at 3-4 true leave stage. All management activities were given as needed till harvesting. Fruits were harvested at breaker stage. Fruits were selectively harvested to maintain uniform color, sizes and fruits without any defects. The selectively harvested fruits were cleaned to remove the dust. Then the fruits were allowed to dry for half an hour by spreading on newspaper over the floor. Initial weight was taken from each variety and packed in the aforementioned packaging materials. Half kilogram tomato fruits per treatment were packed in each packaging materials at room temperature (20-22°C) (Fig. 2).

Fig. 2 - Experimental arrangement at room temperature (20-22°C).

Data collection

Physiological weight loss, pH of tomato fruit juice, decay percentage, color score**,** disease incidence (%), overall acceptability and percentage marketability were collected during the experimental period from the total population at 3 days interval (0, 3, 6, 9, 12, 15 and 18 days).

The physiological weight loss was taken at 3 days interval starting from date of packaging and determined using the methods described by (Workneh *et al.*, 2012). It was determined using sensitive balance (type JD2000-2). The following formula was used to calculate weight loss.

$$\text{Physiological weight loss (\%)} = \frac{\text{Initial weight - final weight}}{\text{Initial weight)}} \times 100$$

The juice content (%) was determined by crashing randomly selected fruits then extracting using juice extractor and calculate juice content as follows:

$$\text{Juice content (\%)} = \frac{\text{Total weight of juice-beaker weight}}{\text{Total weight of fruit}} \times 100$$

pH of tomato fruit juice. Randomly selected fruits from each packaging was extracted using juice extractor and measure with pH meter (Harvard digital pH meter, Model H198103, made from Italy).

Disease incidence (%). The fruits were observed visually for rotting and microbial infection and calculated according to the formula given below (Khruengsai *et al.*, 1991).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

Percentage of marketability. The marketable quality of fruits was subjectively assessed by procedure of Workneh *et al.* (2012) with a slight modification. These descriptive quality attributes were determined subjectively by observing the level of visible mold growth, decay, shriveling, smoothness and shininess of fruits with (15 respondents). A 1-5 rating, with1 = unusable, 2 = usable, 3 = fair, 4 = good, 5 = excellent was used to evaluate the fruit quality. Fruits receiving a rating of 3 and above were considered as marketable. The numbers of marketable fruits was used as a measure to calculate the percentage of marketable fruits during storage. After subjectively assessing the product, it was calculated using the following formula.

$$\text{Marketability \%} = \frac{\text{No. of marketable tomato fruit}}{\text{Total no. of sampled tomato fruit}} \times 100$$

Shelf life. The shelf life was calculated by counting the days required to attain the last stage of ripening, but up to the stage when fruit remained still acceptable for marketing (Rao *et al.*, 2011).

Decay or rotting (%). Decay or rotting was determined by the visual observation. Development of spots on the fruit's skin and softening and rotting of fruits were also being recorded.

Color and overall acceptability score. Color was measured by comparing with the color chart described by Dadzie and Orchard (1997). It was determined by counting (15 respondents) the number of respondents from 0 to 5 scoring. A 1-5 rating with 0 = poor, 2 = fair, 3 = good, and 4 = very good and 5 = excellent and finally the mean data will be analyzed. On the same way, overall acceptability was assessed by the above selected respondents and the result was taken by the 1-5 rating similar with marketability. The predominant colors which were used for evaluation are Green, 2=Breaker, 3=Turning, 4=Pink, 5= Light Red, 6= Red and 7 = Deep Red.

Total soluble solids (TSS). The TSS was determined

following the procedures described by (Waskar *et al.*, 1999). An aliquot of juice was extracted using a juice extractor. A hand refract meter (WAY-2s Abb'e, 0-20 Brixo) was used to determine TSS the refractor meter was washed with distilled water. The refractometer was standardized against distilled water (0 percent TSS) and measure TSS.

Data analysis

The data obtained was statistically analyzed for analysis of variance (ANOVA) using General Linear Model (GLM) using SAS 9.13 version (SAS, 2002).The mean separation was made based on Least Significant Difference (LSD) at 5% level of significance.

3. Results and Discussion

Physiological weight loss

Tomato fruits experience great physiological weight loss if stored under normal conditions without any treatment and safe storage environment (Iqbal *et al.*, 2022). Physiological weight loss of tomato fruits was significantly influenced by the main effect of packaging materials (P<0.001), varieties (P<0.01) and interaction effect (P<0.01) of packaging materials and varieties at 18th days of storage. The result showed that, the highest physiological weight loss (79.88%) was recorded from control (without packaging) followed by Open carton (74.70%) from variety Roma VF (Table 2). The lowest Physiological weight loss (32.72%,) was recorded from variety Oval red with perforated plastic package, which was lower than the weight loss of tomato fruits subjected to all other packaging materials.

In the current investigation, the highest weight loss observed in tomato fruits at the control treatment may be due to the higher respiration rate exercised. The result is supported by the findings of Sinha *et al.* (2019), in which maximum weight loss was recorded from control, without packaging materials. In addition, another group of researchers also report-

Table 2 - Interaction effects of packaging materials and varieties on physiological weight loss and decay percentage (at 9, 12, 15 and 18 days) of tomato at Mersa during 2021 cropping season

Variety	Packaging materials	Physiological weight loss				Decay percentage			
		9	12	15	18	9	12	15	18
Woyno	OC	29.73 c	41.37 d	59.99 d	68.89 d	20.00 c	40.00 d	80.00 c	20.00 a
	CC	23.59 e	31.51 g	49.06 f	58.02 f	0.00 c	0.00 d	46.67 c	80.00 c
	OWB	26.77 d	36.38 e	51.83 e	60.10 e	20.00 a	20.00 c	60.00 a	80.00 c
	CWB	25.04 e	34.64 f	48.73 g	56.59 g	0.00 c	20.00 c	40.00 d	80.00 c
	PPB	13.19 g	19.69 h	27.75 h	32.72 h	0.00 c	0.00 d	20.00 e	60.00 d
	NPPB	2.83 h	40.09 d	58.14 d	67.14 d	0.00 c	0.00 d	60.00 a	100.00 a
	C	40.96 a	56.03 a	68.55 a	79.47 a	0.00 c	0.00 d	60.00 a	100.00 a
Oval red	OC	31.89 b	41.94 d	61.45 d	71.27 d	20.00 a	40.00 a	60.00 a	80.00 c
	CC	24.24 e	32.95 g	47.70 g	55.33 g	0.00 c	20.00 c	46.67 c	100.00 a
	OWB	30.75 c	40.45 d	58.63 d	68.01 d	0.00 c	20.00 c	40.00 d	80.00 c
	CWB	23.10 e	31.17 g	45.02 g	52.21 g	20.00 a	20.00 c	40.00 d	80.00 c
	PPB	15.86 f	21.56 h	31.23 h	36.23 h	0.00 c	0.00 d	20.00 e	60.00 d
	NPPB	2.34 h	40.09 d	58.14 d	67.44 d	20.00 a	20.00 c	33.33 d	86.67 c
	C	42.33 a	57.57 a	68.65 a	79.63 a	0.00 c	26.67 b	53.33 b	80.00 c
Roma VF	OC	32.78 b	44.44 b	64.40 b	74.70 b	0.00 c	20.00 c	60.00 a	93.33 b
	CC	24.50 e	33.32 g	48.54 g	56.29 g	20.00 a	40.00 a	60.00 a	93.33 b
	OWB	31.55 c	42.90 c	62.21 c	72.15 c	0.00 c	40.00 a	60.00 a	86.67 c
	CWB	23.74 e	31.99 g	46.34 g	53.76 g	0.00 c	20.00 c	60.00 a	86.67 c
	PPB	16.88 f	21.87 h	31.71 h	36.78 h	0.00 c	0.00 d	20.00 e	60.00 d
	NPPB	2.62 h	40.34 d	58.49 d	67.64 d	0.00 c	20.00 c	60.00 a	93.33 b
	C	42.65 a	58.00 a	68.86 a	79.88 a	6.67 b	20.00 c	60.00 a	100.00 a
LSD (0.05)		2.03	2.31	3.99	4.39	4.15	4.152	10.99	10.17
Significant level		4.40 **	7.01 **	15.87 **	22.83 **	320.65 ***	276.19 ***	250.79 ***	143.92 ***
CV (%)		5.21	3.77	4.52	4.28	5.21	3.77	4.52	4.28

* significant at P≤0.05; ** highly significant at P≤0.01; *** very highly significant at P≤0.001; means with the same letter (s) within a column are not significantly different at 5% level of significance.

ed that the highest weight loss was recorded for tomato stored in ambient atmosphere without packaging (control) (Sualeh *et al.*, 2016). The highest weight loss from control and open carton may be due to faster metabolism at higher temperature, increased cell wall degradation and higher membrane permeability leading to exposure of cell water for easy evaporation (Yao *et al.*, 2020).The lowest physiological weight loss might be due to the fact that, polyethylene plastic protects the fruits from adverse conditions by avoiding mechanical damage, reducing moisture loss, providing beneficial modified atmosphere and preventing pilferage (Sinha *et al.*, 2019). Similarly, Hailu *et al.* (2014) reported that weight loss of fruits in polyethylene bags was far low than from unpackaged fruits. Lower weight loss of packaged fruits could be due to slow rate of transpiration and prevention of excessive moisture loss.

Decay percentage

Packaging materials vary in their tendency to reduce decay percentage of tomato fruits (Olveira-Bouzas *et al.*, 2021). Decay percentage was significantly influenced by packaging materials (P<0.001), tomato varieties (P<0.05) and their interactions (P<0.001). The highest decay percentage (100 %) was obtained from both Roma VF and Woyno without packaging. It was followed by non-perforated polyethylene plastic and closed carton. However, the lowest decay percentage (20 %) was recorded from variety Woyno with open carton followed by Oval red (60%) with perforated plastic (Table 2). It is clearly identified that decay percentage increased with the storage time for all storage methods and ripening stages. This result is line with the work of (Moneruzzaman *et al.*, 2009). Tomatoes at light red stage showed rapid deterioration. Total deterioration of the fruit was recorded from closed carton with variety Oval red, non-perforated polyethylene plastic with variety of Woyno, and Control both from variety Roma VF and Woyno on the 18th days of storage period.

Disease incident percentage

Disease incident was significantly influenced by packaging materials (P<0.001), tomato varieties (P<0.001) and their interactions (P<0.001). Maximum disease incident (100%) was recorded from both variety Woyno and Oval red using non-perforated polyethylene plastic, followed by the same packaging materials from Roma VF variety (93.33%).

Disease incidence was found only in fruits with non-perforated polyethylene plastic and closed carton packaging materials (Table 3). This could be due to inadequate air flow in non-perforated plastic and the accumulation of water from fruit respiration, which creates an environment conducive to fungi growth. After 12 days, the variety Woyno under closed cartons also started disease incidence while Roma VF in non-perforated polyethylene plastic increased by 20 % while all other packaging materials showed no incidence of disease. This result is in line with the findings of (Bautista-Baños *et al.*, 2008). The highest disease occurrence may be due to the increasing of moisture content in the storage of both closed carton and non-perforated polyethylene plastic packages. The water accumulation inside the packaging is high because of limited movement and exchange of air which can result in the occurrence of fungal disease.

Color

Color was significantly influenced by packaging materials (P<0.001) and their interactions (P<0.05). During the storage period, there was a general change of tomato fruit colour from breaker to deep red. The highest color change was observed in control followed by open carton and wooden box. Variation of skin color was due to variety and packaging material. In comparison of tomato fruit variety color change from the breaker to deep red, Roma VF variety showed highest loss of greenness, while it was lowest in Woyno (Table 3). This result is in line with the work of Tigist *et al.* (2013), who reported that variety Roma VF showed faster rate of loss of greenness while it was slower for Melkasalsa that during normal ripening of tomato fruit, tissue colour changes from green through orange to red, which coincides with ethylene biosynthesis and a climacteric rise in respiration. The color acceptability of control fruits is shorter as compared to other packaging materials (between 3 to 18 days of their storage), that is, in the range of very good to excellent especially perforated plastic packed tomato fruits. This change was due to the action of treatments on the fruits as polyethylene packaging (perforated) helps the color retention as described by (Ashenafi and Tura, 2018).

pH of tomato fruit juice

A significant variation in pH of tomato fruit juice was observed due to the main effect of packaging materials (P<0.001), varieties (P<0.01) and their interactions (P<0.001). The control had the highest pH value (4.23) in all varieties of tomato fruit fol-

Table 3 - Interaction effects of packaging materials and varieties on disease incidence percentage and color (at 9, 12, 15 and 18 days) of tomato at Mersa during 2021 cropping season

Variety	Packaging materials	Disease incidence				Color			
		9	12	15	18	9	12	15	18
Woyno	OC	0.00 c	0.00 d	0.00 d	0.00 e	5.33 c	6.00 a	7.00 a	7.00 a
	CC	0.00 c	6.67 c	20.00 b	20.00 c	5.00 c	6.00 a	7.00 a	7.00 a
	OWB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	6.00 a	7.00 a	7.00 a
	CWB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	6.00 a	7.00 a	7.00 a
	PPB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	5.67 a	6.67 b	7.00 a
	NPPB	0.00 c	20.00 b	40.00 a	100.00 a	4.00 d	5.00 b	6.00 c	6.67 b
	C	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	5.67 a	7.00 a	7.00 a
Oval red	OC	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	6.00 a	7.00 a	7.00 a
	CC	0.00 c	0.00 d	0.00 d	20.00 c	5.00 c	6.00 a	7.00 a	7.00 a
	OWB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	6.00 a	7.00 a	7.00 a
	CWB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	6.00 a	7.00 a	7.00 a
	PPB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	5.67 a	6.67 b	7.00 a
	NPPB	20.00 a	20.00 b	40.00 a	100.00 a	4.33 d		6.00 c	6.67 b
	C	0.00 c	0.00 d	0.00 d	0.00 e	5.33 c	6.00 a	7.00 a	7.00 a
Roma VF	OC	32.78 b	44.44 b	64.40 b	74.70 b	5.00 c	6.00 a	7.00 a	7.00 a
	CC	0.00 c	0.00 d	6.67 c	6.66 d	5.00 c	6.00 a	7.00 a	7.00 a
	OWB	0.00 c	0.00 d	0.00 d	0.00 e	5.33 c	6.00 a	7.00 a	7.00 a
	CWB	0.00 c	0.00 d	0.00 d	0.00 e	5.67 b	6.00 a	7.00 a	7.00 a
	PPB	0.00 c	0.00 d	0.00 d	0.00 e	5.00 c	6.00 a	7.00 a	7.00 a
	NPPB	20.00 a	40.00 a	40.00 a	93.33 b	4.00 d	5.00 b	5.00 d	6.00 c
	C	0.00 c	0.00 d	0.00 d	0.00 e	6.00 a	6.00 a	7.00 a	7.00 a
LSD (0.05)		0.43	0.42	2.08	4.16	0.46	0.36	0.29	0.29
Significant level		57.49 ***	66.67 ***	44.4 ***	27.51 ***	0.20 **	0.17 **	0.17 ***	0.06 *
CV (%)		12.96	6.11	18.04	15.60	5.52	3.73	2.66	2.60

* significant at P≤0.05; ** highly significant at P≤0.01; *** very highly significant at P≤0.001; means with the same letter(s) within a column are not significantly different at 5% level of significance.

lowed by Open wooden box in varieties Roma VF (4.18) and Oval red (4.15).Whereas lowest pH value were obtained in packaged fruits treated with closed carton from Woyno variety (3.1) (Table 4).

The result showed that pH values ranging from 3.10-4.23 from all variety and packaging materials after 12 days of storage period. Generally, the pH of fruits increases as fruits undergo ripening. This might be due to citric acid in tomato juice, with pH of fruit normally between 4.0 and 4.5 (Anyasi *et al.*, 2016). The higher pH of fruits under ambient storage condition could be associated with the utilization of acids for catabolism of sugar at faster rate. High storage temperature leads to faster respiration rate. The lower pH values of packaged fruits could be explained by the relatively reduced respiration rate in the package can inhibit loss of organic acids.

Fruit juice content

Fruit juice content and functional properties of tomato and other vegetables is likely to be affected by the type of storage condition and packaging materials (Alenazi *et al.*, 2020). In the current experiment, a significant variation in fruit juice content was observed due to the main effect of packaging materials (P<0.001) and varieties (P<0.01). PPB had highest fruit juice content (93.70%) on Oval red tomato fruit varieties and followed by both OC and control (92.37%) under variety Roma VF. Whereas, the lowest juice content was recorded from OWB (85.26)

and CWB (85.44) packaging materials on Woyno variety followed by NPPB (88.26%) on Oval red variety (Table 4). Similar findings were reported by (Gebeyehu, 2018). The variation of juice content may be due to differences in packaging materials which can affect the firmness and metabolic processes. The variation in juice content of tomato fruit among varieties might be due to their genetic differences.

Total soluble solids (°Brix)

Total soluble solid (TSS) is one of the quality parameters in fruits and vegetables (Mesa *et al*., 2022). In the present research, TSS was significantly influenced by packaging materials (P<0.001), varieties (P<0.001) and interaction effect of packaging methods and varieties (P<0.01). During the 12 day of storage period the TSS was range from 3.65 to 5.11°Brix (Table 4). The result showed that, the highest TSS (5.11) was recorded from variety Roma VF under the control followed by Oval red variety. The lowest TSS (3.65) was recorded from variety Woyno using non-perforated polyethylene plastic (Table 4). The result agrees with the work of Sualeh *et al.* (2016).

Table 4 - Interaction effects of packaging materials and varieties on Juice content, PH and TSS of tomato at Mersa during 2021 cropping season

Variety	Packaging materials	Storage period (days)		
		Juice content	pH	TSS
Woyno	OC	89.30 d	4.01 f	4.65 f
	CC	88.39 e	3.10 h	4.53 g
	OWB	85.44 e	4.05 f	4.59 g
	CWB	85.26 e	3.98 f	4.40 h
	PPB	90.29 d	4.12 e	4.08 i
	NPPB	88.96 d	3.75 g	3.65 j
	C	92.03 c	4.23 a	4.87 c
Oval red	OC	91.65 c	4.04 f	4.36 i
	CC	90.83 d	4.02 f	4.45 j
	OWB	90.51 d	4.15 d	4.27 i
	CWB	91.69 c	3.98 f	4.38 i
	PPB	93.70 a	4.12 e	4.01 j
	NPPB	88.26 e	4.06 f	4.02 j
	C	92.37 b	4.23 b	5.01 b
Roma VF	OC	92.37 b	4.02 f	4.69 e
	CC	91.46 d	4.04 f	4.36 i
	OWB	91.91 c	4.18 c	4.57 g
	CWB	91.31 d	3.93 f	4.41 h
	PPB	92.03 c	4.13 e	4.52 g
	NPPB	91.92 c	3.26 h	4.02 j
	C	92.04 c	4.23 a	5.11 a
LSD (0.05)		3.21	0.16	0.37
Significant level		7.13 *	0.07 ***	0.14 **
CV (%)		2.07	2.48	4.68

* significant at P≤0.05; ** highly significant at P≤0.01; *** very highly significant at P≤0.001; means with the same letter(s) within a column are not significantly different at 5% level of significance.

The variation in TSS might be due to advancement of fruit ripening, packaging materials and variety of tomato fruit. Total soluble solids (°Brix) of control and treated tomato fruits showed that they increased as the ripening proceeds. The lowest TSS value of non-perforated polyethylene plastic may be due to slowing down of respiration and metabolic activity. Whereas the highest TSS in the control may be due to high respiration and metabolic activity rise ripening process as result increasing TSS. In this regard, the view of Kumar *et al.* (2022) is noteworthy that the slower respiration also slows down the synthesis and use of metabolites resulting in lower TSS due to the slower change from carbohydrates to sugars which result in retardation of the ripening process.

The changes in TSS content that occur during ripening are correlated with the hydrolytic shifts in starch concentration after harvest. Using sugars as a respiration substrate, the TSS content of fruits could decrease with time storage due to increased temperature and biosynthesis processes or polysaccharide degradation at maturity (Azene *et al.*, 2014).

Overall acceptability (score)

Overall acceptability was significantly influenced by packaging materials (P<0.001), tomato varieties (P<0.01) and their interactions (P<0.001). The present study revealed that under Woyno variety, non-perforated polyethylene plastic and control fruits showed, lower overall acceptability as compared to other packaging materials and varieties at 18 days of storage period (Table 5). At the later stage of ripening (18 days), Non Perforated polyethylene plastic and control fruits showed a lower overall acceptability scores (0-3). Oval red and Roma VF varieties of tomato fruits under perforated plastic showed better overall acceptability followed by both carton packed and wooden box packaging (Table 5).

The results led to a conclusion that the main reason behind this improvement was due to the prevention of fruit from decay organism and the fruit will have stored reserve which is protected from adverse

Table 5 - Interaction effects of packaging materials and varieties on overall acceptability and marketability percentage (at 9, 12, 15 and 18 days) at Mersa during 2021 cropping season

Variety	Packaging materials	Overall acceptability				Marketability percentage			
		9	12	15	18	9	12	15	18
Woyno	OC	4.00 d	3.00 d	1.00 c	0.00 c	80.00 d	60.00 c	20.00 c	0.00 c
	CC	4.00 d	3.00 d	1.00 c	0.00 c	80.00 d	60.00 c	20.00 c	0.00 c
	OWB	3.00 f	4.00 b	1.00 c	0.00 c	60.00 f	60.00 c	20.00 c	0.00 c
	CWB	4.00 d	4.00 b	1.00 c	0.00 c	80.00 d	60.00 c	20.00 c	0.00 c
	PPB	5.00 a	4.00 b	2.00 b	1.00 a	100.00 a	80.00 a	40.00 a	20.00 a
	NPPB	3.00 f	2.00 f	0.00 e	0.00 c	40.00 g	20.00 f	0.00 d	0.00 c
	C	4.00 d	2.00 f	1.00 c	0.00 c	60.00 f	40.00 e	20.00 c	0.00 c
Oval red	OC	4.00 d	3.00 d	1.00 c	0.00 c	80.00 d	60.00 c	20.00 c	6.67 b
	CC	4.00 d	3.00 d	2.00 b	0.00 c	80.00 d	60.00 c	20.00 c	0.00 c
	OWB	4.00 d	2.33 e	1.00 c	1.00 a	80.00 d	53.33 d	20.00 c	0.00 c
	CWB	4.00 d	3.00 d	2.00 b	0.00 c	73.33 e	60.00 c	20.00 c	0.00 c
	PPB	5.00 a	4.00 b	3.00 a	1.00 a	93.33 b	80.00 a	26.66 b	0.00 c
	NPPB	3.00 f	1.67 f	0.00 e	0.00 c	60.00 f	20.00 f	0.00 d	0.00 c
	C	4.00 d	3.00 d	1.00 c	0.00 c	73.33 e	66.67 b	20.00 c	0.00 c
Roma VF	OC	4.00 d	3.00 d	2.00 b	1.00 a	80.00 d	60.00 c	20.00 c	0.00 c
	CC	3.67 e	3.00 d	1.00 c	0.00 c	73.33 e	40.00 e	20.00 c	0.00 c
	OWB	3.67 e	3.33 d	1.00 c	0.00 c	73.33 e	40.00 e	20.00 c	0.00 c
	CWB	4.33 c	3.67 c	0.67 d	0.00 c	86.67 c	60.00 c	20.00 c	0.00 c
	PPB	4.67 b	4.33 a	3.00 a	0.67 b	93.33 b	80.00 a	40.00 a	20.00 a
	NPPB	3.00 f	2.00 f	1.00 c	0.00 c	60.00 f	20.00 f	20.00 c	0.00 c
	C	3.33 f	2.00 f	1.00 c	0.00 c	66.67 f	40.00 e	0.00 d	0.00 c
LSD (0.05)		0.46	0.59	0.21	0.21	11.74	5.87	4.15	4.15
Significant level		0.22 **	0.61 ***	0.71 ***	0.33 ***	144.97 **	170.37 ***	222.22 ***	69.84 ***
CV (%)		6.83	11.63	9.92	56.69	8.80	6.59	13.68	79.37

* significant at P≤0.05; ** highly significant at P≤0.01; *** very highly significant at P≤0.001; means with the same letter(s) within a column are not significantly different at 5% level of significance.

condition (Yahaya and Mardiyya, 2019). From breaker to turning stage, the colour of fruits changed from poor to fair by showing not more than 30% of surface as not green in colour. When stage advances from pink to pink-red, the colour of all fruits was in the range of good to excellent. This result is in conformity with the work of (Priyankara *et al.*, 2017). Up to 9th days Roma VF tomato variety exhibited best color and consumer acceptability, followed by Oval red were as Woyno scores low consumer acceptability.

Marketability percentage

Marketability percentage was significantly influenced by packaging materials (P<0.001), tomato varieties (P<0.05) and their interactions (P<0.001). At 9th days of storage period the highest percentage marketability (100%) was obtained from Woyno tomato fruit varieties using perforated polyethylene plastic followed by the same packaging materials (93.33%) from both Roma VF and Oval red tomato fruit varieties (Table 5). The lowest marketability percentage was obtained from non-perforated polyethylene plastic (40%) of Woyno tomato fruit Variety followed by both Roma VF and Oval red (60%). When the storage period increase in storage period (18 days) tomato fruits packed only with perforated polyethylene plastic (20%) from both Roma VF and Woyno tomato fruit varieties followed by Oval red tomato fruit varieties using open carton packaging materials (6.67%).

The difference in marketability of tomato fruits

was due to packaging materials, varieties of tomato fruits, and also percentage of decayed fruits and disease incidence obtained from non-perforated polyethylene bags. The present result is in line with the study of Haile and Safawo (2018) who reported that, packaging of climacteric fruits in low density polyethylene bags delay ripening and softening, and hence improves marketability. These beneficial effects can be explained by the modified atmosphere created inside the package as well as the reduction in water loss. Lower respiration and ethylene production rates, due to modification of atmospheric gases inside the package could be the possible reason to extend the storage life of fruits (Islam *et al.*, 2022).

4. Conclusions

Tomatoes are prone to careless handling and packaging throughout local manufacturing. As a result, there is a significant postharvest loss of tomato fruits at every stage, from harvest to consumption. The fruit must therefore be handled properly after harvest in order to improve its protection and shelf life. With this context in mind, the goal of this study was to evaluate the impact of packaging material on the quality and shelf life of tomato fruit varieties after harvest.

The physiological weight loss, percentage of decay, color score, general acceptability, TSS, pH, incidence of disease, variety, marketability, and shelf life of tomato fruits were all significantly influenced by packaging. Due to a faster respiration rate, the control had the greatest weight reduction. On perforated plastic, the weight reduction was accompanied by an accelerated water loss. Perforated plastic showed the highest marketability and degradation percentage. The Woyno variety under non perforated plastic had the highest illness incidence and the highest juice content (93.37%) of any type of plastic. Roma VF variety beneath perforated plastic had the highest TSS. When employing perforated plastic, followed by open cartons and hardwood boxes, the storage term for tomato fruit was significantly lengthened.

Under perforated plastic, the tomato variety with a longer shelf life was both Roma VF and Oval red. According to the findings of this study, tomato fruits packaged in low density polyethylene bags with perforations had a longer shelf life and better quality. However, a follow-up study in multiple locations and across seasons is necessary to substantiate this advice. There should be further research done using package types and materials that are not covered in this study.

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Evaluation of viability and germination of pollen grains of three local caprifig cultivars and their effect on some characteristics of fig fruits (*Ficus carica* L.)

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Key words: Caprifig, *Ficus carica*, flower stigma extract, pollen germination.

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Abstract: This research was carried out in fig fields in the village of Kafar-Jales, in the north of Syria. The analyses were conducted in the laboratories of the Faculty of Agriculture at Idlib University during the 2022 farming season, to evaluate the viability and germination of three local varieties of Caprifig (Bunduqi, Azraq, Panjani). The morphological characteristics, the date of the exodus of insects (*Blastophaga psenes*) from male fruits, the quantity of pollen, and the percentage of germination and viability were studied on four nutritive media at a temperature of 27°C. The measurements were taken after 24 h, 48 h, and 72 h. These three pollinators were used to pollinate three varieties of edible figs (white Satehi, Safrawi, and Habashi) to study the effect of pollen source on the productive characteristics of edible fig fruits. The result showed significant superiority of the Bunduqi cultivar over the rest of the cultivars in traits of the fruit's early ripening, pollen quantity, and date of departure of insects. The Azraq cultivar is found to be superior to Bunduqi and Panjani cultivars in the germination rate of pollen at all stages of the experiment and in all used media. The addition of stigma flower extract in culture media increased germination percentage by 35% for all studied Caprifig cultivars. The addition of 10% sucrose in the cultivation environment increased the percentage of pollen germination by 20%. The cultivars that were inoculated with the Panjani pollinator outperformed the characteristics of the length, diameter, and weight, demonstrating that the fruit's quality characteristics are affected by the genotype of the pollinator used. The results of this research can be beneficial for both fig growers and plant breeders, as they help to select the best pollinators and contribute to the development and improvement of the quality of the cultivated fig fruits.

1. Introduction

The fig was one of the first fruit trees domesticated during the Stone Age (Zohary and Spiegel-Roy, 1975). The results of a study of (Çalışkan and Dalkılıç, 2022) clearly showed that the southern regions of Turkey were one of the original centers of figs.

Figs spread in many areas due to their adaptation to different climates and soils (Mars, 2003). The fig tree is mainly found in the Mediterranean region because it is well adapted to the climatic conditions affecting this region (Ighbareyeh *et al*., 2018), and is one of Syria's most important fruit trees. Idlib governorate is at the forefront in production and the number of trees, with 40% of the cultivated area at the level of Syria (Syrian Statistical Group, 2021), where many female varieties are planted whose fruits are eaten fresh or dried. In addition, the caprifig trees are used for pollination purposes, which are residence to the pollinating fig wasp (*Blastophaga psenes*). *Ficus carica* L. belongs to the Moraceae family. The species contains two sexual forms: the male fig (caprifig) and the female fig (edible). The male fig produces pollen as it is male in practice, but at the same time, it contains female flowers with male flowers, which are functionally hermaphrodites. On the other hand, the female (edible) fig has only long-style female flowers that are monosexual (Stover *et al*., 2007). Fig pollen is carried by the fig wasp (*Blastophaga psenes* L.) that develops with the fig tree (Kjellberg *et al*., 1987).

In Syria, there are two different groups of figs. The first group is the male fig (caprifig), which spreads in many regions of the world, and its types are very close to each other (Condit, 1947). The fruits of this species are inedible and contain two types of flowers: male pollen-producing (near the ostiole), and female short-stylet (in two-thirds of the lower cavity). The wasp insect develops inside the tuberous flowers. This type produces three crops annually (Valdeyron and Lloyd, 1979; Stover *et al*., 2007; Flaishman *et al*., 2008). Summer profichi and its fruits pollinate the types of figs that need pollination. The Mammoni crop is used in the fall, and the Mammi crop is used in the winter (Anjam *et al*., 2017). The second group is the edible fig (Smyrna), which produces edible fruits with real seeds. This type contains long-style female flowers, which need to be pollinated by the pollen-bearing fig wasp from the profichi crop of caprifig to give fruits if pollination occurs (Armstrong, 2006). The fruit contains seeds inside, but in the absence of pollination, the inflorescences fall (Armstrong, 2006). This type of fig produces two crops per year, the Breba crop that ripens in early summer. In addition, the second (main) crop ripens at the beginning of autumn (Valdeyron and Lioyd, 1979). *Ficus carica* produces inflorescences called Syconia. The flowers are unisexual, either male, female, or gal flowers (female short-style), and the fruits are borne in the axilla of the leaf (Andersen, and Crocker, 2009; Aytürk, 2019).

The process of pollination in figs is called caprification, and the profichi crop is used for this process. Profichi crop produces much more pollen than the other two crops, and for a high-quality profichi crop, it is preferable to have a high pollen germination rate and a high amount of pollen produced (Balci *et al*., 2001). The research was conducted to study the characteristics of fruits and pollen for different genotypes of the caprifig. It was noted that there are discrepancies between them (Ilgın *et al.*, 2007; Çalışkan and Yaman, 2016; Çalışkan *et al*., 2021). Pollen viability, germination rate, and pollen quantity affect the yield of inoculated fruit trees. Different plants' pollen requires various growing media such as water, sugar solution, inorganic salts, and vitamins for successful germination (Stanley and Linskens, 2012. In a study conducted by Ilgın *et al*. (2007), it was found that pollen did not germinate at all on a medium without sucrose and increasing sucrose concentrations to 20% improved the percentage of pollen germination. To further improve the germination rate, several concentrations of H_3BO_3, KNO_3, and GA3 were added to media containing 20% sucrose. The germination rates of some caprifig pollen grains were higher than 70% with the addition of 0.050% H_3BO_3, followed by 0.025% KNO_3, and the germination rates of caprifig pollen were higher with the addition of these chemicals than sucrose 20% alone. Germination of caprifig pollen was increased to more than 70% by adding stigma exudates from long-style female flowers to the planting medium (Awamura *et al*., 1995). In a study by Ilgın *et al*. (2007), pollen viability was higher than that of pollen germination. This result was consistent with previous studies (Pearson and Harney, 1984; Bolat and Pirlak, 1999; Stanley and Linskens, 2012). Vego and Miljković (2012) found that the best conditions for pollen germination of caprifig were in a medium containing 3% sucrose and 0.01% boric acid solution at a temperature of 30°C in the dark. The selection of pollen of good quality and quantity is important in fig orchards, as several studies indicate that the caprifage variety used can influence the quality traits of fig fruits (Rahemi and Jafari, 2005; Gaaliche *et al*., 2011; Pourghayoumi *et al.*, 2012).

In this research, we will test the viability and germination of pollen grains in three cultivars of wild figs spread in northwestern Syria to determine the most efficient of these pollinators for female (edible) fig orchards. We will also study the effect of pollen

source on some characteristics of female (edible) fig fruits resulting from using these pollinators.

2. Materials and Methods

Plant materials

Three cultivars of caprifig have been studied in the Kafar-Jales area (5 km northwest of Idlib city). Year 2022, rainfed cultivation system, and the cultivars are Bunduqi, Azraq, Panjani (Fig. 1).

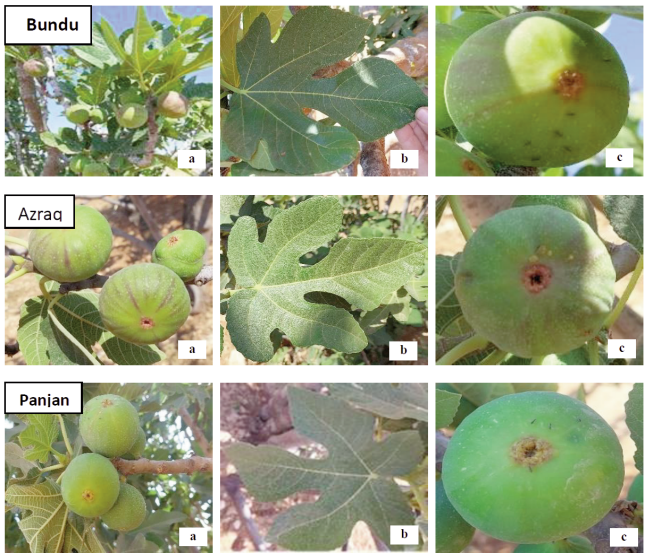

Fig. 1 - Fruits placement on the branch (a), and the shape of the leaves (b) and fruits (c) in Bunduqi, Azraq and Panjani caprifig.

The following characteristics were studied, which are the same characteristics that were studied by Çalışkan *et al.* (2017) on 60 Turkish caprifig cultivars. For each cultivar, these characteristics were studied:

a. number of fruits on the branch;
b. fruit diameter (cm);
c. fruit length (cm);
d. date of the expulsion of the *Blastophaga* insect from the fruits.
e. amount of pollen grains in male flowers.
f. pollen viability;
g. percentage of germination of pollen;
h. timeline of pollen germination rate evolution.

Three varieties of female (edible) figs, widely spread in the study area and economically important for fig growers, were selected: Habashi, white Satehi, and Safrawi (Fig. 2).

To study the quality of fig fruits, the same specifications that were studied in the research of (Gaaliche *et al*. (2012) were studied, where 20 fruits for each variety were studied as follows:

a) fruit length (cm);
b) fruit diameter (cm);
c) fruit weight (g);
d) total soluble solids percentage, TSS (%).

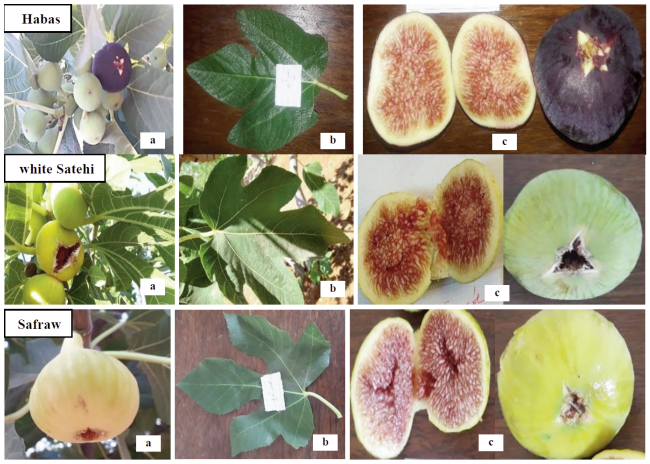

Fig. 2 - Fruits placement on the branch (a), leaves (b), and cross-section and full fruit (c) of Habashi, white Satehi and Safrawi female cultivars.

Study the specifications of the Caprifig fruits

The fruits of caprifigs of the three varieties (Bunduqi, Azraq, Panjani) were harvested during the Profichi crop's emergence in June. The fruits were cut crosswise and placed on paper plates in the laboratory (cool and dry) to collect pollen. Pollen grains were taken from ten separate fruits for each variety using a cylinder gradient and we compared the varieties among them.

Pollen viability test

Pollen viability was tested using methylene blue dyes. The colored pollen grains, after adding methylene blue dye, were considered to live, while the non-colored pollen grains were considered dead. So, 100 pollen grains were counted, the percentage of colored pollen grains was calculated, and the process was repeated three times for each sample.

Pollen germination test

Germination rate was studied after 24, 48, and 72 h of cultivation in media (A, B, C, and D). 0.5 (g) of pollen was taken and grown in each petri dish. The pollen grain was considered germinated if the pollen tube length was greater than the diameter of the pollen grain. The exploding pollen grains were also ignored and were not considered germinated, which

was previously applied in a similar study (Khan and Perveen, 2008). The germination rate of pollen of the caprifig cultivars was studied on medium A (5% sucrose + 1 ppm boric acid + 1% agar), medium B (10% sucrose + 1 ppm boric acid + 1% agar), medium C (5% sucrose + 1 ppm boric acid + 1% agar + extract of stigma female flowers), and medium D (10% sucrose + 1 ppm boric acid + 1% agar + extract of stigma female flowers). The readings were taken 24 h, 48 h, and 72 h after culture. The pollen of the three varieties was grown in the cultivation media and incubated at a temperature of 27°C.). Gaaliche *et al*. (2013) had previously used these mediums to study caprifig pollen germination in a previous study.

Study the effect of pollen source on the characteristics of the edible fig fruits

The study was done on the fruits of the main crop, except Breba, because Breba falls due to the absence of pollinating insects, because it appears in April. So, sixty fruits of each female variety (white Satehi, Safrawi, and Habashi) were isolated at the beginning of their formation by cotton fabric bags that allow air to enter, and the diameter of the holes in them is smaller than the fig wasp insect. When insects started to emerge from the studied caprifig cultivars (Bunduqi, Azraq, Panjani), we took the fruit of the pollinated variety and put it in the bag with the female fruit of the studied varieties so that each female variety was inoculated with the three pollinators (each pollinator separately) at a rate of 20 fruits from each pollinator (20 replicates). The fruits were isolated for two weeks to ensure that the required pollinator was pollinated. The fruits were harvested in stages, so that the fruits are taken when they reach the stage of maturity, and the entire harvest period is during the month of August.

Experiment design and statistical analysis

Factorial experiment in completely randomized design (CRD) was used in the distribution of the experiment's coefficients. The data were statistically analyzed using the GenStat program, and L.S.D values were taken at a significance level of 5% and 1% for field and laboratory readings, respectively.

3. Results and Discussion

Maturity date (when insects begin to emerge) and characteristics of caprifig syconia

The results obtained (Table 1) show that the cultivar Bunduqi was early concerning the date of the emergence of insects. Insects began to emerge from the syconia of Bunduqi on 01/06/2022, with a difference of more than a week from the two other varieties (Panjani and Azraq), which makes this variety suitable for pollinating the female fruits of the early fig varieties, especially the first fruits that appear on the branch (where they fall in the absence of pollination). The emergence of insects from the syconia of this variety continued for a week. As for the cultivars Panjani and Azraq, the beginning of the release of insects in them was 8/6/2022 and lasted for 10 days, which makes them suitable for pollination of the edible fig variety whose flowers appear in the middle of flowering time.

As for pollen, it was arbitrarily classified s few in the flowers of the cultivar Azraq, abundant in the cultivar Panjani and very abundant in the cultivar Bunduqi (Table 1).

Pollen viability of caprifig cultivars

Pollen viability was studied in caprifig cultivars Azraq, Bunduqi, and Panjani using methyl blue dye

Table 1 - Specifications of the Syconia of the Profichi crop

Cultivar	Number of Syconia*	Syconia length (cm)	Syconia diameter (cm)	Pollen quantity	Syconia ripening (Insect exit)
Azraq	6.00 a	4.14 c	4.58 c	few	Medium (08-06-2022)
Bunduqi	6.20 a	5.12 b	5.40 a	very abundant	Early (01-06-2022)
Panjani	6.50 a	5.88 a	4.84 b	abundant	Medium (08-06-2022)
Mean	6.23	5.05	4.94		
L.S.D. (5%)	0.71	0.18	0.17	-	-
C.V. %	12.40	3.90	3.80	-	-

The presence of the same letter in each column indicates that there are no significant differences between the items.
* The quantity (few, abundant) is a random unit to compare pollen collected in a drum of different cultivars).

(Fig. 3). Table 2 shows the results of the pollen viability study for the three cultivars.

Table 2 shows no significant differences in the percentage of pollen viability among the three studied cultivars Panjani, Azraq, and Bunduqi. The highest percentage of viability was observed in the cultivar Panjani (99.67%) and the lowest percentage of viability in the cultivar Bunduqi (97.33%).

Study of the percentage of germination of pollen of caprifig on media (A, B, C, and D)

24 h after planting. Table 3 shows the superiority of the cultivar Azraq in the percentage of pollen germination over the two cultivars Panjani and Bunduqi after 24 h of cultivation on different media. The averages of germination rates were 20.85%, 9.63%, and 8.94%, respectively. There were no significant differences in the germination characteristics of the two cultivars Panjani and Bunduqi. The highest germination percentage was obtained in media B and D, with values of 16.35% and 16.16%, without significant differences. The two media B and D were significantly superior to medium C, which gave a germination rate of 11.80%. This was significantly superior to medium A, which gave a germination rate of 8.24%. As for the interaction between the caprifig variety and the medium used in cultivation, the interaction of the Azraq variety with the medium B and the interaction of the blue medium D variety achieved the highest germination rate of 24.69% and significantly superior to the other interactions (without significant differences between these two reactions). In comparison, the interaction of the Bunduqi cultivar with medium A achieved the lowest germination percentage of 5.02% after 24 h of pollen cultivation.

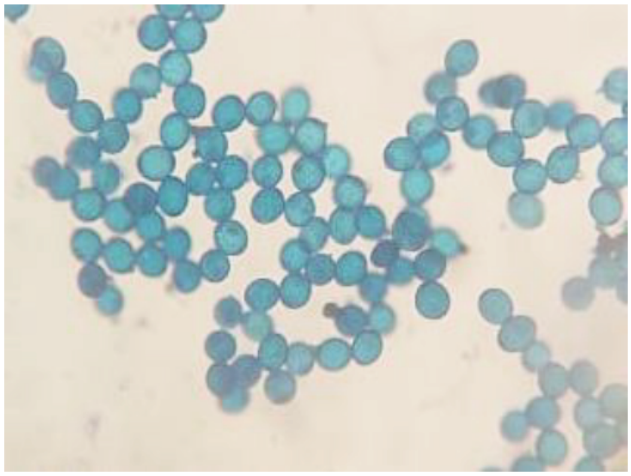

Fig. 3 - Pollen viability test using methylene blue.

Table 2 - Pollen viability percentage using methylene blue dye

Cultivar	Dye methylene blue (%)
Azraq	98.67
Bunduqi	97.33
Panjani	99.67
C.V.%	3.40
L.S.D. (1%) = Cultivar	3.47

Table 3 - Germination of pollen grains of caprifig cultivars after 24 h of planting on media (A, B, C, and D)

Cultivar	Medium				Average
	A	B	C	D	
Azraq	12.69	25.69	20.35	24.69	20.85 a
Bunduqi	5.02	13.02	5.02	12.69	8.94 b
Panjani	7.02	10.35	10.02	11.12	9.63 b
Average	8.24 c	16.35 a	11.80 b	16.16 a	13.14
C.V.%	7.28				
L.S.D. (1%) = Cultivar	1.10				
L.S.D. (1%) = Medium	1.27				
L.S.D. (1%) = (Cultivar x Medium)	2.20				
Pr. Cultivar	<0.001				
Pr. Medium	<0.001				
Pr. (Cultivar x Medium)	<0.001				
F. calculated. Cultivar	584.57				
F. calculated. Medium	148.35				
F. calculated. (Cultivar x Medium)	21.97				

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

Figure 4 show the pollen germination of Panjani, Azraq, and Bunduqi cultivars after 24 h of cultivation on media (A, B, C, and D).

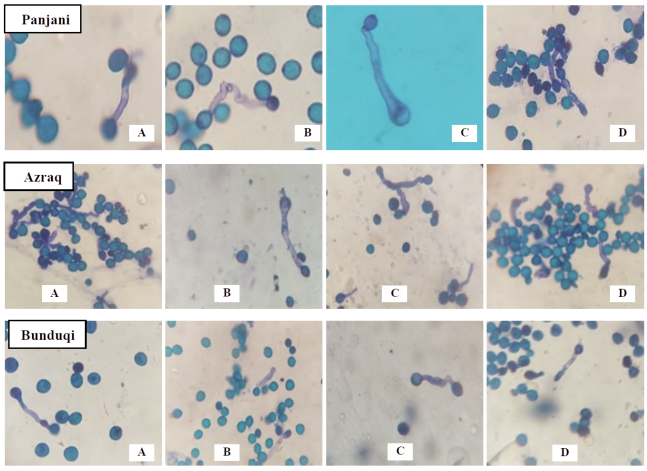

Fig. 4 - Germination of 'Panjani', 'Azraq' and 'Bunduqi' pollen after 24 h of cultivation on media (A, B, C, and D).

48 h after planting. The results of the statistical analysis of the percentage of pollen germination caprifig cultivars (Table 4) show that the Azraq cultivar was superior in the germination percentage of pollen grains to the two cultivars Panjani and Bunduqi after 48 h of planting on media (A, B, C, and D), with an average germination rate of 33.32%. There were no significant differences between the two cultivars Bunduqi and Panjani, whose germination rates reached 24.99% and 23.85%, respectively. As for the media used in cultivation, medium D (10% sucrose + extract of the stigma of female flowers) significantly outperformed the other cultivation media and gave a germination rate of 33.81%. Medium C (5% sucrose + extract of the stigma of female flowers) was significantly superior, with a germination rate of 29.88% over medium B (10% sucrose), which achieved a germination rate of 25.32%, and in turn significantly outperformed medium A (5% sucrose), which came in the last rank, with a germination rate of 20.55%.

Regarding the interaction between the caprifig variety and the medium used in cultivation, the interaction of the Azraq variety with medium D achieved the highest germination percentage, which amounted to 40.32%, and significantly outperformed all other interactions. On the other hand, the interaction of the Bunduqi cultivar with medium A achieved the lowest germination rate, which was 16.99% after 48 h of pollen cultivation.

Figure 5 show the pollen germination of Panjani, Azraq, and Bunduqi cultivars after 48 h of cultivation on media (A, B, C, and D).

72 h after planting. Table 5 displays the superiority of the cultivar Azraq in the percentage of pollen germination on the two cultivars Panjani and Bunduqi after 72 h of cultivation on media (A, B, C, and D), with an average germination rate of 64.16%.

Table 4 - Germination of pollen grains of caprifig cultivars after 48 h of planting on media (A, B, C, and D)

Cultivar	Medium				Average
	A	B	C	D	
Azraq	25.32	36.32	31.32	40.32	33.32 a
Bunduqi	16.99	18.66	31.66	32.66	24.99 b
Panjani	19.32	20.99	26.66	28.44	23.85 b
Average	20.55 d	25.32 c	29.88 b	33.81 a	27.39
C.V.%	5.37				
L.S.D. (1%) = Cultivar	1.69				
L.S.D. (1%) = Medium	1.96				
L.S.D. (1%) = (Cultivar x Medium)	3.39				
Pr. Cultivar	< 0.001				
Pr. Medium	< 0.001				
Pr. (Cultivar x Medium)	< 0.001				
F. calculated. Cultivar	584.57				
F. calculated. Medium	148.35				
F. calculated. (Cultivar x Medium)	21.97				

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

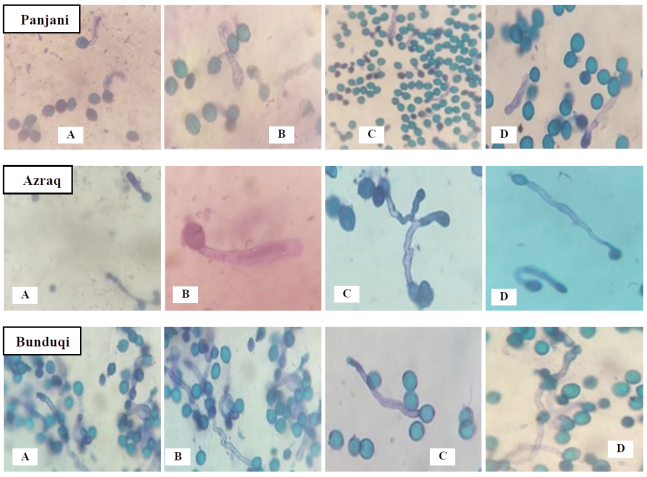

Fig. 5 - Germination of 'Panjani', 'Azraq' and 'Bunduqi' pollen after 48 h of cultivation on media (A, B, C, and D).

sucrose), which achieved a germination rate of 46.00%. Medium A (5% sucrose) came in the last rank with a percentage of germination of 38.33%. Regarding the interaction between the caprifig cultivar and the medium used in cultivation, the two interactions, the Azraq cultivar with the medium D, and the blue cultivar with the medium B were significantly superior to the other interactions, with a percentage of germination of 69.66% and 68.66%, respectively, without significant differences between them. The interaction of the Bunduqi cultivar with medium A achieved the lowest germination rate of 23.66% after 72 h of pollen cultivation. Figure 6 show the germination of pollen of cultivars Panjani, Azraq, and Bunduqi after 72 h of cultivation on media (A, B, C, and D).

Table 5 - Germination of pollen grains of fig cultivars after 72 h of cultivation in media (A, B, C, and D)

Cultivar	Medium				Average
	A	B	C	D	
Azraq	53.66	68.66	64.66	69.66	64.16 a
Bunduqi	23.66	30.33	50.66	55.33	40.00 c
Panjani	37.66	39	54.33	57.7	47.17 b
Average	38.33 d	46.00 c	56.55 b	60.90 a	50.44
C.V.%	2.62				
L.S.D. (1%) = Cultivar	1.76				
L.S.D. (1%) = Medium	1.52				
L.S.D. (1%) = (Cultivar x Medium)	3.04				
Pr. Cultivar	< 0.001				
Pr. Medium	< 0.001				
Pr. (Cultivar x Medium)	< 0.001				
F. calculated. Cultivar	584.57				
F. calculated. Medium	148.35				
F. calculated. (Cultivar x Medium)	21.97				

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

The cultivar Panjani was also significantly superior to the cultivar Bunduqi, which came in the last rank, the germination rates for both were 47.17% and 40.00%, respectively. Concerning the agricultural media, all media differed significantly among themselves in the characteristic of the percentage of germination of pollen. Medium D (10% sucrose + extract of the stigma of female flowers) significantly outperformed the other cultivation media, with a germination rate of 60.90%. Medium C (5% sucrose + extract of the stigma of female flowers) was significantly superior, with a germination rate of 56.55% over Medium B (10%

Evolution of germination rate during the experimental time

The previous results show that the percentage of pollen germination increased for all the studied caprifig cultivars with the progression of the experiment. While the average percentage of germination for all tested cultivars after 24 h was 13.14%, the percentage increased to 27.39% after 48 h of planting, and it reached 50.44% after 72 h. The cultivar Azraq gave the best germination rate and outperformed the other cultivars in all stages of the experiment. The percentage of germination in this cultivar

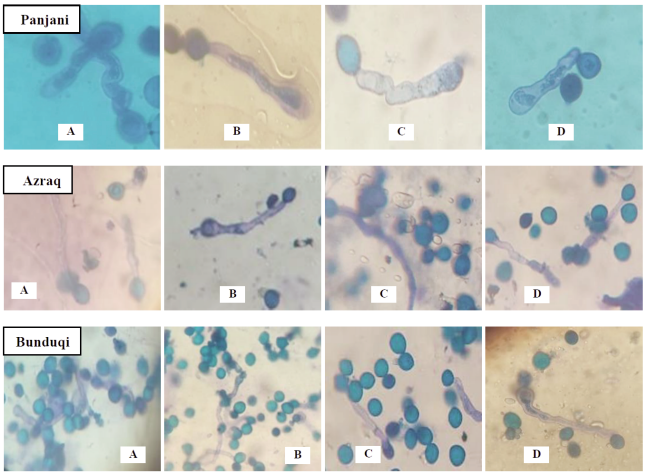

Fig. 6 - Germination of 'Panjani', 'Azraq' and 'Bunduqi' pollen after 72 h of cultivation on media (A, B, C, and D).

increased from 20.85% to 33.32% and then reached 64.16% after 24 h, 48 h, and 72 h of planting, respectively. As for the cultivar Panjani, the germination percentage increased from 9.63% to 24.99% and then to 47.17% after 24 h, 48 h, and 72 h, respectively. The percentage of germination in the Bunduqi cultivar increased from 8.94% to 23.85% and then to 40% after 24 h, 48 h, and 72 h, respectively. There were no significant differences in the germination percentages of Panjani and Bunduqi at the beginning of the experiment (after 24 h to 48 h of cultivation), but the cultivar Panjani gave a higher percentage of germination and outperformed the variety at the end of the experiment (after 72 h of cultivation).

This study is consistent with the study of (Ilgin *et al.*, 2007; Gaaliche *et al.*, 2013), where the germination rate increased with time and reached the highest value 72 h after planting.

Regarding the media used for cultivation, the percentage of germination increased in all media used in culture (A, B, C, and D) with the progression of the experiment. Also, media C and D (containing the extract of the female stigma) significantly outperformed the media A and B (which did not contain the extract of the female stigma) in most reading times and for all the studied caprifig cultivars. The average percentage of germination in media containing extract of the female stigma was 34.85%, while the average percentage of germination in media that did not contain extract of the female stigma was 25.80% (an increase in the percentage of germination was about 35% when the extract of the female stigma was added to the pollen culture medium). This proves the positive role of adding the extract of the long-style female stigma flowers in raising the percentage of pollen germination of caprifig varieties and increasing the growth of the pollen tube in them. These results are consistent with several previous studies (Ilgin *et al.*, 2007; Awamura *et al.*, 1995), where pollen germination from caprifig was increased to more than 70% when long-style stigmas were added to the pollen culture medium.

Media B and D (containing 10% sucrose) significantly outperformed media C and D (containing 5% sucrose) in most reading times and for all studied caprifig cultivars, where the average percentage of germination in media containing 10% sucrose was 33.09%. The average percentage of germination in media containing 5% sucrose reached 27.56% (an increase in germination percentage was about 20% when the level of sucrose in the cultivation medium increased from 5% to 10%). This confirms the importance of increasing the percentage of sucrose in the cultivation medium of pollen of caprifig varieties to raise the percentage of germination. This is consistent with the results of Ilgin *et al.* (2007), who found that increasing the concentration of sucrose to 20% led to an increase in the percentage of pollen germination but increasing the percentage of sucrose above 25% in the cultivation medium led to a decrease in the percentage of pollen germination. However, our results do not agree with the results of Zeybekoglu *et al.* (1997), who found that the best germination rate of pollen of caprifig cultivars was at a concentration of 5% of sucrose.

Effect of pollen type on the fruit quality of edible fig cultivars

Fruit length

Table 6 displays the significant superiority of the pollinator Panjani over the two pollinators, Bunduqi and Azraq, in the characteristic of the fruit length of the edible fig variety, with a value of 4.62 cm. The Bunduqi pollinator was significantly superior to the Azraq pollinator, with a fruit length of 4.23 cm and 4.11 cm, respectively. As for the edible fig cultivars, the Safrawi and Habashi fig cultivars (without significant differences) were significantly superior to the white Satehi cultivar, and the fruit length values were 4.89 cm, 4.80 cm, and 3.26 cm, respectively. In terms of the effect of the interaction of the pollinated variety with the edible fig variety on the characteristic of the length of the fruit, the interaction of the caprifig cultivar Panjani with Safrawi achieved the highest

Table 6 - Effect of the pollinated variety on the characteristic of fruit length in edible fig varieties

Caprifig	Fruit length (cm)			Average
	Habashi	White Satehi	Safrawi	
Azraq	4.59	3.36	4.39	4.11 c
Bunduqi	4.77	3.08	4.83	4.23 b
Panjani	5.04	3.35	5.46	4.62 a
Average	4.80 a	3.26 b	4.89 a	4.32
C.V.%	3.60			
L.S.D. (1%) = Caprifig	0.06			
L.S.D. (1%) = Edible fig	0.05			
L.S.D. (1%) = (Caprifig x Edible fig)	0.10			
pr. Caprifig	<.001			
pr. Edible fig	<.001			
pr. (Caprifig x Edible fig)	<.001			
F. calculated. Caprifig	169.59			
F. calculated. Edible fig	2031.91			
F. calculated. (Caprifig x Edible fig)	64.10			

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

value (5.46 cm) and significantly outperformed all other interactions. The interaction of the caprifig variety Bunduqi with white Satehi was in the last place with a fruit length of 3.08 cm.

Fruit diameter

The pollinator Panjani was significantly superior to the pollinated Bunduqi and Azraq pollinators in terms of the diameter of the fruit of the female variety, with a value of 5.34 cm (Table 7). Also, the Bunduqi pollinator was significantly superior to Azraq pollinator with a fruit diameter of 4.88 cm and 4.75 cm, respectively. As for the edible fig cultivars, the Habashi was significantly superior to Safrawi and white Satehi cultivars. Safrawi was significantly superior to the white Satehi cultivar, with a fruit diameter of 5.78 cm, 4.76 cm, and 4.43 cm, respectively. In terms of the effect of the interaction of the inoculated variety with the edible fig variety on the characteristic of the diameter of the fruit, the interaction of

Table 7 - Effect of the inoculated variety on the fruit diameter in edible fig varieties

Caprifig	Fruit diameter (cm)			Average
	Habashi	White Satehi	Safrawi	
Azraq	4.59	3.36	4.39	4.11 c
Bunduqi	4.77	3.08	4.83	4.23 b
Panjani	5.04	3.35	5.46	4.62 a
Average	4.80 a	3.26 b	4.89 a	4.32
C.V.%	3.60			
L.S.D. (1%) = Caprifig	0.06			
L.S.D. (1%) = Edible fig	0.05			
L.S.D. (1%) = (Caprifig x Edible fig)	0.10			
pr. Caprifig	<.001			
pr. Edible fig	<.001			
pr. (Caprifig x Edible fig)	<.001			
F. calculated. Caprifig	169.59			
F. calculated. Edible fig	2031.91			
F. calculated. (Caprifig x Edible fig)	64.10			

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

the caprifig variety Panjani with the edible fig variety Habashi significantly outperformed all other interactions, with a fruit diameter of 6.08 cm. In contrast, the interaction of the caprifig cultivar Azraq with Safrawi came at the last position with a fruit diameter of 4.18 cm.

Fruit weight

From Table 8, it appears that the pollinator Panjani was significantly superior to the pollinated Bunduqi and Azraq in the fruit weight of the edible fig variety, with a value of 53.83 g. The Bunduqi pollinator was significantly superior to the Azraq pollinator with a fruit weight of 48.90 g. While the Azraq pollinator came in last place with a fruit weight of 47.32 g. As for the edible fig cultivars, the Habashi fig cultivar was significantly superior to Safrawi and white Satehi cultivars. Safrawi also outperformed the white Satehi cultivar (the fruit weight values of the edible fig cultivars were 56.73 g, 49.07 g, and 44.28 g, respectively). Regarding the interaction of the caprifig pollinated variety with the edible fig variety and its effect on the characteristic of the weight of the fruit, the interaction of the caprifig cultivar Panjani with the edible fig cultivar Habashi significantly outperformed all other interactions, the weight of the fruit in this interaction was 59.40 g. The interaction of the caprifig cultivar Azraq with the edible fig cultivar Safrawi gave the lowest value of the fruit weight (42.90 g).

Total soluble solids percentage

Table 9 shows no significant differences between caprifig varieties in their effect on the TSS % in the edible fig fruits inoculated with these pollinators. The values were 22.35%, 22.22%, and 21.32% after inoculation with Panjani, Azraq, and Bunduqi cultivars, respectively. Thus, no effect of the inoculated variety was observed on the TSS percentage in the edible fig fruits. As for the female cultivars, the White Satehi cultivar was significantly superior to both cultivars Habashi and Safrawi in terms of the TSS %, which amounted to 25.35%, 20.78%, and 19.75%, respectively (and there were no significant differences between the two cultivars Habashi and Safrawi). Regarding the effect of the interaction of the caprifig pollinator variety with the edible fig variety on the characteristic of the TSS % in the fruits of the pollinated varieties, the interaction of the caprifig cultivar Panjani with the edible fig cultivar White Satehi significantly outperformed all other interactions, with a TSS % of 26.45%. In the latest place, the interaction of the caprifig cultivar Bunduqi with the edible fig cultivar Habashi came with a TSS % of 18.55%.

Our results agreed with the findings of Pourghayoumi *et al.* (2012), who indicated the significant effect of the pollen source on the length of the fruit in the edible fig varieties pollinated with these pollinators. However, our results did not match the results of same study regarding the effect of the pollen source on the TSS % feature.

Table 8 - Effect of the pollinated variety on the characteristic of the weight of the fruit in the edible fig varieties

Caprifig	Fruit weight (g)			Average
	Habashi	White Satehi	Safrawi	
Azraq	55.45	43.6	42.9	47.32 c
Bunduqi	55.35	43.2	48.25	48.90 b
Panjani	59.4	46.05	56.05	53.83 a
Average	56.73 a	44.28 c	49.07 b	50.03
C.V.%	5.00			
LSD (1%) = Caprifig	0.90			
LSD (1%) = Edible fig	0.90			
LSD (1%) = (Caprifig x Edible fig)	1.57			
pr. (Caprifig x Edible fig)	<.001			
pr. Caprifig	<.001			
pr. Edible fig	<.001			
F. calculated. Caprifig	109.85			
F. calculated. Edible fig	376.28			
F. calculated. (Caprifig x Edible fig)	26.87			

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

Table 9 - Effect of the inoculated variety on the TSS % in the fruits of edible fig varieties

Caprifig	TSS (%)			Average
	Habashi	White Satehi	Safrawi	
Azraq	21.85	24.75	20.05	22.22 a
Bunduqi	18.55	24.85	20.55	21.32 a
Panjani	21.95	26.45	18.65	22.35 a
Average	20.78 b	25.35 a	19.75 b	21.96
C.V.%	9.40			
LSD (1%) = (Caprifig x Edible fig)	1.28			
LSD (1%) = Caprifig	0.74			
LSD (1%) = Edible fig	0.74			
pr. (Caprifig x Edible fig)	<.001			
pr. Caprifig	0.013			
pr. Edible fig	<.001			
F. calculated. Caprifig	4.50			
F. calculated. Edible fig	126.35			
F. calculated. (Caprifig x Edible fig)	11.09			

The presence of the same letter in the same column or the same line indicates that there are no significant differences between the transactions.

4. Conclusions

This research demonstrated that the cultivar Bunduqi was the earliest caprifig cultivar in terms of fruit ripening, the abundance of pollen, and the time of the release of Blastophaga insects. Therefore, it is recommended to use it in pollinating early female fig varieties. Also, the pollen viability was very high in all studied cultivars, and there were no significant differences between cultivars in this trait. Furthermore, the Azraq cultivar significantly outperformed Panjani and Bunduqi cultivars in pollen germination percentage in all phases of the experiment and in all media used in cultivation. Adding stigma flower extract in cultivation media increased the germination rate by 35% for all studied caprifig cultivars. Furthermore, increasing the proportion of sucrose (10%) in the cultivation medium increased the pollen germination percentage by 20% for all cultivars of caprifig (compared to 5% sucrose). Finally, the caprifig variety used in pollination had a clear effect on the weight, diameter, and length of the fruit in the pollinated edible fig varieties. Consequently, the Panjani variety was superior to the rest of the pollinators in improving these traits. The results of this research are important for both fig growers and plant breeders, as they help to select the best pollinators and contribute to the development and improvement of the quality of the cultivated fig fruits.

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Short note

Use of the biostimulant Retard Cherry® as a strategy to delay blooming period in sweet cherry trees

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Abstract: Spring frosts are a limiting factor in sweet cherry production in central-southern Chile. Sweet cherry trees cv. 'Regina' and 'Sweetheart' were studied to evaluate the effect of foliar application of a biostimulant (Retard Cherry®) prior to leaf fall on the bloom delay, fruit set, fruit drop, yield and quality. Data were compared to a non-product control. The study was conducted in the Maule Region, Chile. Results showed that the use of Retard Cherry® delayed full bloom by 6-8 days between cultivars compared to the control; however, there was no delay in the harvest date. The climatic conditions favored high fruit set (37%-49%) and low fruit drop (63%-70%) between cultivars in both treatments. Regarding fruit quality, no differences in size, soluble solids concentration and color were observed with the product, but a decrease in firmness were observed for 'Regina'. These results show that Retard Cherry® is an effective tool in delaying bloom, providing trees with more favorable climatic conditions for pollination and fruit set.

1. Introduction

Sweet cherry (*Prunus avium* L.) is a fruit tree of temperate climate, native to Asia Minor (Iezzoni *et al.,* 2017). Its cultivation has been widely distributed in Mediterranean climate countries such as Turkey, Italy and Spain, in the United States and in Middle Eastern countries such as Iran (Bujdosó and Hrotkó, 2017). In the southern hemisphere, Chile is the main exporter, with a volume of 350 thousand tons in the 2020/21 season in a cultivated area of 48,960 ha (iQonsulting, 2021; ODEPA, 2021).

Emergence from recess and regulation of blooming time in deciduous fruit trees involves a combined process of winter cold and spring warmth accumulation (Fadón *et al.,* 2020). In sweet cherry trees, temperature conditions during the cold accumulation phase are estimated to be the main driver of bloom (Fadón *et al.,* 2021). In mild winter areas, insufficient cold accumulation can cause delayed bloom, floral malformations, and low fruit production in the trees. In contrast, when high cold accumulation accompanied by spring warmth occurs in this period, bloom may be

advanced and flower opening concentrated, increasing the risk of spring frost damage (Herrero *et al.,* 2017), which can damage flowers and buds (Miranda *et al.,* 2005) and generate a large decrease in crop production and profitability (Kaya *et al.,* 2021). Moreover, low temperatures can affect blooming synchrony between variety and pollinizer, limiting pollinator activity, delaying pollen tube development and fruit set (Guo *et al.,* 2015).

Climate change in the near future is expected to generate warmer springs with greater thermal fluctuations, which may alter plant phenology and increase the risk of spring frost damage in species of temperate climate (Augspurger, 2013). Some tools used to reduce spring frost damage are overhead sprinkler irrigation, wind towers with heaters and heated macro-tunnels. However, these technologies have a high implementation cost and are not always effective enough in control (Yuri *et al.,* 2017).

A complementary strategy to avoid frost damage in fruit trees is to delay tree bloom, shifting it to a period with greater climatic stability, more favorable for pollination and fruit set (Liu and Sherif, 2019). Plant growth regulators evaluated in stone fruits can extend bud dormancy (Durner and Gianfagna, 1991), delay bloom (Ebel *et al.,* 1999) or synchronize bloom with another variety, as well as delay harvest (Basak *et al.,* 1998). In some cases, however, the application of these products can cause flower abscission, low fruit set and yield (Crisosto *et al.,* 1990; Liu and Sherif, 2019). On the other hand, the use of foliar biostimulant, as sustainable alternatives to plant growth regulators, could increase flower bud resistance to winter cold and delay bloom, without detrimental effects on fruit production.

This study aimed to evaluate the effect of foliar application of the biostimulant Retard Cherry® on bloom delay before leaf fall as well as on fruit set, fruit drop and fruit quality in 'Regina' and 'Sweetheart' sweet cherry trees in central Chile.

2. Materials and Methods

Plant material and experimental site

The study was conducted during the 2018/19 growing season in two commercial orchards of sweet cherry (*Prunus avium* L.) located in San Clemente, Maule Region, Chile (35°32' S, 71°27' W, 230 m a.s.l.), at less than 5 km between them. In one orchard, 'Regina' sweet cherry trees on 'Gisela-6' rootstock were evaluated; they were planted at 4.0 × 1.8 m in 2015. In the other, 'Sweetheart' sweet cherry trees on 'Colt' rootstock were evaluated; they were planted at 5.0 × 2.5 m in 2010. Both cultivars were trained in Central Leader. Orchards management were carried out according to commercial standards in the region. Seasonal environmental conditions are summarized in Table 1.

Climatic data was recorded by an automatic weather station Vantange 2 (Davis Instruments, Hayward, CA, USA) near the orchards. During the

Table 1 - Environmental conditions 2018, San Clemente, Chile

Variable	Annual	May 1-Jul 31	Aug 1-Dec 25	October
Air temperature (°C)				
Mean	13.6	7.4	13.7	13.3
mean maximum	21.4	13.3	21.3	20.2
mean minimum	7.3	3.1	7.2	7.6
Maximum	35.6	25.2	35.4	26.4
Minimum	-5.1	-5.1	-5.1	1.5
Relative humidity (%)				
mean minimum	46.1	66.8	42.1	44.5
Precipitation (mm)	493	234	205	37.8
Solar radiation (MJ m^{-2})	5.718	573	2.677	829
Chill Hours	1.433	857		
Chill Units (Utah)	478	1.308		
GDH	72.507		30.59	6.65
GDD (base 10°C)	1.807		697	123

Note: Chill Hours: Weinberger, 1950. Chill Units (Utah): Richardson *et al.*, 1974. GDH: Anderson and Seeley, 1992. GDD: Stanley *et al.*, 2000).

blooming period, environmental conditions favourable to bee activity were calculated, defined as the number of hours per day with air temperature higher than 15°C and solar radiation higher than 300 W m^{-2}.

Experimental design

The experimental design was a randomized by complete block divided into two treatments (5,000 m^2 per treatment). The treatments were: (1) control without product; (2) foliar application of Retard Cherry® (AM Ecological S.A., Chile). The product was applied twice at doses of 1.0 and 0.5 L/ha, prior to 50% leaf drop: for 'Regina' on March 26 and April 9; for 'Sweetheart' on March 15 and 30. Applications were made with a conventional hydro-pneumatic sprayer (Parada SpA, Santiago, Chile) with a spray volume of 1,200 L/ha. All measurements were made in 10 replicates, each consisting of two branches per replicate, trees per replicate, considering three edge rows per side.

Bloom delay

Blooming evolution (%) was determined weekly from stage 'first white' to 'full bloom', counting the number of open flowers per date. The full bloom date was defined when 80% had flowered. Bloom delay was determined by subtracting the days between the full bloom date of the control and that of the treatment.

Fruit set, fruit drop and yield

Fruit set and fruit drop were monitored on the same branches studied at bloom. Fruit set (%) was evaluated 20 days after the full bloom evaluation by counting the number of fruits formed in relation to the total number of flowers per branch. Fruit drop (%) was determined by the number of fruits that did not reach harvest in relation to the number of fruits formed. Yield (kg/tree) was also determined from individual trees.

Fruit quality

Harvest date was determined on the basis of fruit color. Evaluation of weight (g), diameter (mm), color, firmness, and soluble solids concentration (SSC) considered a sample of 50 fruits per treatment. Color was determined visually by scale (light red = 1, red = 2, mahogany red = 3, dark mahogany = 4 and black = 5). Firmness (g mm^{-1}) was measured with a FirmTech II texturometer (BioWorks Inc, Wamego, USA). SSC (°Brix) was measured on the same fruits with a digital refractometer (Atago, PLAS-BX/ACID5, Japan).

Statistical analysis

The data obtained underwent an analysis of variance (ANOVA) and the means were compared with the Tukey test (P≤0.05). When necessary, a transformation of the data was carried out. Analysis was performed with the Statgraphics Centurion XVI program (Warrenton, Virginia, USA) and the figures were generated using SigmaPlot 10 software (WPcubed GmbH, Germany).

3. Results

Sweet cherry trees treated with Retard Cherry® showed a 6-8 day delay in the full bloom date with respect to the control, with a greater delay in the case of 'Regina' (Table 2, Fig. 1). Although the application of Retard Cherry® delayed bloom, it had no effect on harvest date (Table 2).

A similar level of fruit set was maintained

Table 2 - Effect of foliar application of Retard Cherry® on the date of phenological stage in sweet cherry trees 'Regina' and 'Sweetheart'

Cultivar/treatments	80% full bloom	Harvest
'Regina'		
Control	09-Oct	25-Dec
Retard Cherry	17-Oct	25-Dec
'Sweetheart'		
Control	27-Sep	21-Dec
Retard Cherry	02-Oct	21-Dec

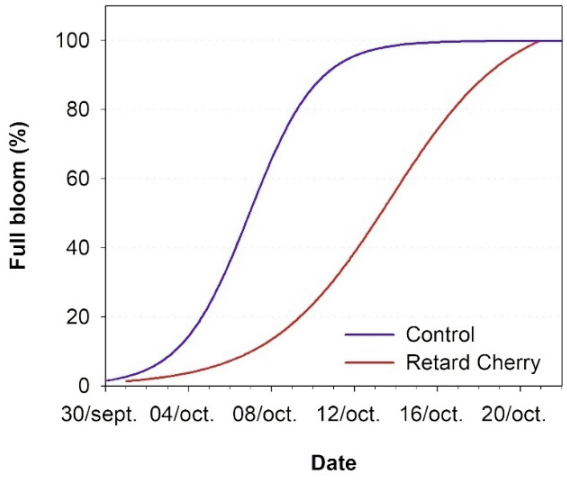

Fig. 1 - Effect of foliar application of Retard Cherry® on blooming dynamic in sweet cherry trees cv. Regina.

between treatments with an average of 49% for 'Regina' and 37% for 'Sweetheart' (Table 3). Even though fruit drop was numerically higher in the treatment with Retard Cherry®, it was not statistically significant, which is reflected in tree yields, with a mean of 8.6 kg/tree for 'Regina' and 14 kg/tree for 'Sweetheart' (Table 3).

The 'Regina' sweet cherry trees treated with Retard Cherry® showed 18% lower firmness than the control, with no change in fruit color, while 'Sweetheart' showed a higher incidence of fruit with lower color, although this was not noticeable to consumers. Fruit weight, diameter, and SSC were not affected by treatment (Table 4).

Table 3 - Effect of foliar application of Retard Cherry® on fruit set, fruit drop and fruit yield in 'Regina' and 'Sweetheart' sweet cherries

Cultivar/treatments	Fruit set (%)	Fruit drop (%)	Yield (kg/tree)
'Regina'			
Control	50 a	65 a	8.6 a
Retard Cherry	47 a	75 a	8.6 a
P-value	0.45	0.16	-
'Sweetheart'			
Control	33 a	ND	14.0 a
Retard Cherry	41 a	63	14.0 a
P-value	0.41	-	-

Means in a column followed by the same letter do not differ statistically, according to Tukey test (P≤0.05). n= 10/treatment. ND= no detected.

Table 4 - Effect of foliar application of Retard Cherry® on fruit quality in 'Regina' and 'Sweetheart' sweet cherries at harvest

Cultivar/ treatments	Weight (g)	Diameter (mm)	Color (1-5)	Flesh firmness (g mm^{-1})	SSC (°Brix)
'Regina'					
Control	12 a	28 a	4.0 a	231 a	20 b
Retard Cherry	12 a	28 a	4.4 a	189 b	21 a
P-value	0.41	0.31	0.09	0.00	0.01
'Sweetheart'					
Control	13 a	29 a	4.2 a	274 a	21 a
Retard Cherry	12 b	29 a	3.6 b	271 a	20 b
P-value	0.04	0.46	0.01	0.80	0.00

Means in a column followed by the same letter do not differ statistically, according to Tukey test (P≤0.05). n = 50/treatment.

4. Discussion and Conclusions

The results of fruit set are in concordance with those of Raffo and Curetti (2021) who reported a delay of up to 10 days in leaf emergence and full bloom of several sweet cherry cultivars in Rio Negro, Argentina. A greater assimilation and subsequent transport of reserves to the plant, prior to leaf fall, would allow an adequate dormancy and a more homogeneous bloom, effect that could be favored by the foliar application of biostimulants in autumn.

Fruit set in sweet cherry trees is normally low, and highly dependent on pollen availability and climatic conditions during and after pollination (Hedhly *et al.,* 2007). In Chile, sweet cherry growers use the following scale of fruit set intensity: high 34-40%; medium 15-20%; low 8-10% (C. Tapia, pers. comm, October 11, 2021).

Sagredo *et al*. (2017) found that the effective pollination period in 'Regina' sweet cherry cultivars was about 5 days post anthesis, with the highest fruit set levels occurring 2-3 days post anthesis. Similarly, Zhang *et al.* (2018) showed that the peak pollen germination and stigma receptivity of certain sweet cherry cultivars occurred 2-3 days post anthesis under three ambient temperature scenarios. In addition, the pollen tube required at least 48 h to reach the ovule.

Regarding to bee activity, more than 100 bee visits per minute were observed by Koumanov and Long (2017) with proper hive management, temperatures above 18°C and wind speed less than 16 km h^{-1}. On the other hand, Vicens and Bosch (2000) indicated that bee activity (*A. mellifera*) was fully active with air temperature above 14°C and solar radiation greater than 300 W m^{-2}.

The high fruit set obtained in this study could have been favored, among others, by the prevalence of suitable climatic conditions for bee activity during blossom and for fruit set of that season (Table 5; Fig. 2). No frost was observed during blossom in both orchards. Post-bloom environment of the trees treated with Retard Cherry® was more stable, with an average daily mean air temperature about 1-2 °C higher than that measured in the control trees one week earlier.

Table 5 shows the great difference in the conditions for bee flight at blooming between the two cultivars. The application of Retard Cherry, by delaying blooming, had a much greater effect on 'Sweetheart', as it distanced it from the riskiest date of low tem-

Table 5 - Environmental conditions in the seven days since full bloom for both cultivars and treatments

Cultivar/Treatment	Period	Air temperature (°C)			Relative humidity (%)	Days with rainfall	Rainfall (mm)	Bee activity (h) [x]
		mean	max	min				
'Regina'								
Control	9 Oct - 15 Oct	11.8	18.8	5.4	69.4	0	0	30
Retard Cherry	17 Oct -23 Oct	13.8	20.8	7.7	64.4	2	3	37
'Sweetheart'								
Control	2 Sept - 3 Oct	11.0	16.7	6.2	71.8	4	18	17
Retard Cherry	2 Oct - 8 Oct	11.9	19.5	6.1	72.8	1	11	26

[x] Bee activity (hours with air temperature > 15°C and solar radiation > 300 W m^{-2}).

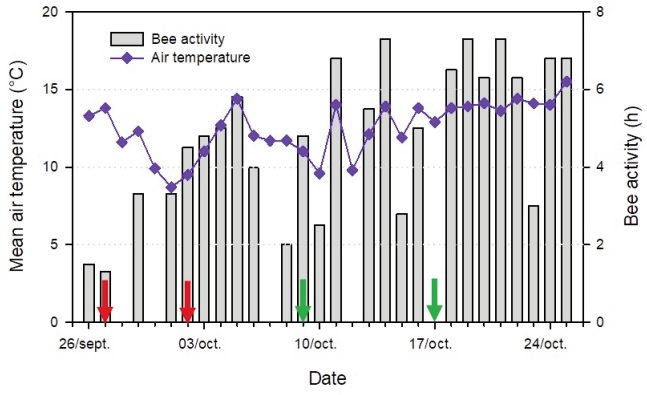

Fig. 2 - Mean daily air temperature and hours favorable for bee activity (>15°C and > 300 W m^{-2}) per day from 26 September to 25 October 2018.
Arrows indicate full bloom date: green for 'Regina' and red for 'Sweet-heart'. The first arrow corresponds to the control for both cultivars.

peratures and frost in early spring; something similar occurred in the case of 'Regina', although with less intensity, as it is a later blooming cultivar.

Harvest date was not affected using Retard Cherry® on sweet cherry trees, although there was evidence of lower fruit firmness on Regina and slightly less coloration on 'Sweetheart' (Tables 2 and 4). The fruit ripening stage is characterized by a rapid increase in weight and size, due to an increase in cell size, leading to a reduction in firmness. Sugar content increases, keeping acids relatively constant; however, color is the one that shows the greatest changes, being a relevant factor in determining the harvest date in sweet cherries (Tudela *et al.,* 2005; Muskovics *et al.,* 2006). In previous studies, Raffo and Curetti (2021) reported that the use of Retard Cherry® showed a marked delay in color development of 'Sweetheart' sweet cherries. The use of GA_3 allows delay harvest in sweet cherry trees and has also proven to increase fruit size, firmness and SSC (Basak *et al.,* 1998; Horvitz *et al.,* 2003; Raffo and Curetti, 2021). Therefore, GA_3 would be a complementary tool to the use of foliar biostimulant Retard Cherry®, to extend the harvest window, with good quality fruit and better prices.

Therefore, it can be concluded that the use of Retard Cherry® on 'Regina' and 'Sweetheart' sweet cherry trees is an effective tool for delaying bloom to avoid frost event and favor conditions for bee flight. No delay in the harvest date was observed. Fruit set and fruit drop percentages were not affected by the treatment. At harvest, fruit from trees treated with Retard Cherry® showed no differences in size and SSC but showed lower firmness for 'Regina' and less color for 'Sweetheart' compared to those harvested from control trees.

Since this study was carried out under specific climatic conditions, further investigations will be necessary to consolidate the results obtained.

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Review paper

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The habit of strawberry flowering is the key for runner propagation, where the photoperiod is the main environmental factor - A review

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Key words: Chilling, division, flowering, *Fragaria x ananassa* Duch, gibberellin, photoperiod, proliferation.

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Abstract: Despite the advancement of tissue culture in strawberry plant propagation, the degree of elite for field cultivation depends on forcing the plant to produce runners. The strawberry flower habit [everbearing (EB), seasonal berry (SB), short-day (SD), long-day (LD), and day-neutral (DN)] defines the method of encouraging the plant to generate runners, since the formation of runners is mostly influenced by genetic factors before being influenced by environmental factors. Stolon production, which occurs as a result of vying for resources under certain environmental circumstances, is the reverse of blossoming. Therefore, any stimulus that encourages stolon formation and vegetative growth limits the development of flower buds, which is necessary for elite propagation. Long photoperiod, temperature, chilling hour, or cold storage, and plant growth regulators (PGR) are cited as these variables. Temperature has a significant impact on runner development, although the long daily photoperiod (LD) remains the most crucial component in runner induction. However, when LD interacts with other factors like temperature, cold storage, and gibberellins, its efficiency is increased. Thus, based on the cultivars and the seasonal climate of the geographical location, the best approach for strawberry propagation is identified by optimising the planting date for propagation or adjusting the propagation circumstances.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch) is a commercial crop grown worldwide for its nutritional and health benefits. Strawberries are consumed as fresh fruit or juice, or processed industrially into jam used in a various of desserts such as candy, milk and ice cream. According to the FAO statistics service agency (FAO, 2021), strawberry production has increased significantly over the past half-century (from 1960 to 2021). The total global production in 2021 is estimated to be around 9,175,384.43 t, with a cultivated area of about 389,665 ha. China con-

tributes for approximately 37% to global production, while the Arab nations contribute only 6%, with Egypt, Morocco, and Jordan making up the majority.

The development of strawberry production in various countries depends on the selection of the most suitable cultivars for their annual climate. Vegetative propagation is the ideal technique for strawberry propagation since it retains the mother's characteristics (Li *et al*., 2020). Strawberry nurseries, as a result, play an important role in the expansion of strawberry cultivation within a specific geographical area. It is critical for commercial plant production to select the best factors to stimulate the plant to produce runners.

In this review, the vegetative growth of strawberry plants will be covered as a technique for containing blossoms and encouraging the plant to generate runners. Blossoming and the development of runners are mutually exclusive. Flowers must be controlled in the practical application of runner production, either by eliminating the flowers or by altering the environmental conditions. Long photoperiods (DL) and high temperature (HT) are crucial for promoting stolonization (Smeets, 1955; Smeets and Kronenberg, 1955; Went, 1957; Leshem and Koller, 1965; Smeets, 1980), which is related to increased gibberellin production (Tafazoli and Vince-Prue, 1978). In addition, cold storage of plants promotes the production of runners (Hamano *et al*., 2009; Watanabe *et al*., 2009; Al-madhagi *et al*., 2018). Exogenous application of growth regulators such as gibberellins, cytokinins or their combination supports the development of the runners (Kender *et al*., 1971). Long photoperiods (LD) are the most essential factors for runnering induction in strawberry, but its effectiveness is enhanced by its interaction with other factors such as temperature, cold storage, and gibberellins.

Within this context, the primary goal of this paper is to present an overview of the factors that influence strawberry runner yield.

Strawberry flowering habit and cultivars division

Genetics is the primary component governing strawberry proliferation. Moreover, it pinpoints the best techniques as well as the coefficients of the propagation means. Since *F. ananassa* Duch, the cultivar of the strawberry, is a hybrid plant, the variations in strawberry cultivars may be attributed to variations in its fundamental parents, *F. virginiana* and *F. chiloensis*, each of which has a unique blooming and runnering behaviour. *F. chiloensis* began to bloom before *F. virginiana* and its majority of the leaves stayed evergreen throughout the winter. In the meantime, genotypes of *F. virginiana* seemed to become dormant, and their leaves became brown and withered off in the late fall and winter, outperforming *F. chiloensis* for runner production (Darrow, 1966; Hancock *et al.*, 2003).

The capacity of the bud in cultivars to continue producing inflorescences throughout the growing season accounts for the variation in runner development. According to other researches (Guttridge, 1985; Hytönen and Elomaa, 2011), this is connected to the differentiation of the meristem into a leaf rosette, also known as a branch crown or stolon. A branch crown serves as a platform for inflorescences, whereas a runner is a vegetative, extended shoot with a terminal daughter plant that may be employed for clonal multiplication (Samad *et al*., 2021).

Based on physiological and production features, strawberry plants were split into various classes. As seen in figure 1, each group has a specific function, one for physiological characteristics and the other for productive attributes. Because they influence planting and harvesting dates, as well as seedling reproduction and harvesting techniques, physiological features are important. For both fresh and processed products, the production features (qualitative and quantitative) are essential to satisfy local consumers' needs and exporters' demands.

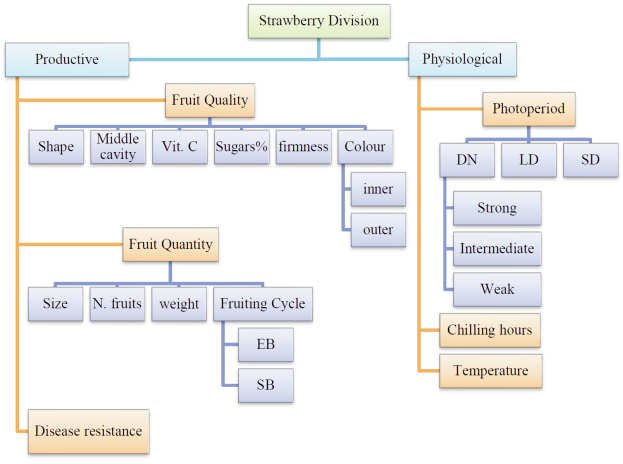

Fig. 1 - A list of different features that can be taken into account to describe a strawberry cultivar and eventually classify it. Strawberry cultivars are classified into physiological and productive traits, with physiological traits playing the most important role in determining runner performance and productivity. SD: short-day, LD: long-day, DN: day-neutral, EB: everbearing, SB: seasonal berry.

Based on how they react to the photoperiod for flower induction, strawberry cultivars are categorised as short-day (SD), long-day (LD), or day-neutral (DN) (Durner *et al*., 1984). The ideal method for plant multiplication, blooming behaviour, and fruit production is determined by this division, which is crucial. In the meanwhile, strawberry cultivars exhibit considerable addiction as a result of interactions between photoperiodic temperature. This link has led to the interchange ability of the two terms when describing strawberry blossoming behaviour (Cai *et al*., 2017).

Based on their respective production periods, strawberry cultivars were divided into two groups: everbearing (EB) and seasonal berry (SB). This division may be thought of as an implementation of actual cultivar behaviour in response to temperature and photoperiod. While certain places of the world may only see one season of production from different strawberry SB kinds, other locations may experience two seasons. As a result, the split of production cycles is inaccurate globally while being based on the same latitude (Table 1).

The major gene that regulates blooming and runner production is called Perpetual Flowering Runnering (PFRU) (Hytönen and Kurokura, 2020). Retentive, day-neutral (DN), continuous flowering, and long-day plants are all examples of everbearing (EB) cultivars (Cai *et al*., 2017). The words "day-neutral" (DN) and "everbearing" (EB) are interchangeable and refer to a physiological insensitivity to day-duration in flower bud initiation and a realistic expectation of strawberry producing. Weak, moderate, and strong day-neutral cultivars can be used to categorise the everbearing strawberry cultivars (Nicoll and Galletta, 1987).

EB cultivars can produce multiple crops throughout the year, regardless of day length, at a significantly higher temperature than seasonal berry (SB) cultivars (Smeets, 1980).

Everbearing (EB) strawberry cultivars, in contrast to SB strawberry cultivars, have been linked to early flowering and initiation at shoot tips, resulting in better crown branching ability (Hytönen and Elomaa, 2011). As a result, EB strawberry cultivars tend to produce few stolons on a large scale (Darrow, 1966; Simpson and Bell, 1989; Dale *et al.*, 1996), and fewer stolons than SB. Since branch crowns are ended by inflorescences, the quantity of branch crowns is essentially correlated with the quantity of inflorescences (Hytönen *et al*., 2004; Tenreira *et al*., 2017).

In the meanwhile, branch crowns that form from buds in the leaf axils of the crowns of mature plants are divided to create economically viable EB strawberry plants. The EB strawberry cultivars are quantitative LD plants at medium temperatures, day neutral only at low temperatures (15°C), and qualitative LD plants at high temperatures (Pedraza *et al*., 2010; Samad *et al*., 2021). Short-day (SD) conditions, as seen in figure 2, cause EB cultivars to stop growing and become dwarfed throughout the summer season (Darrow and Waldo, 1934). For growth, bloom initiation, and stolon formation in EB strawberries, a critical photoperiod of 15 hours at 18°C and 14 hours at 30/25°C day/night temperature is needed (Nishiyama *et al*., 2006; Sønsteby and Heide, 2007).

Seasonal strawberry blooms (SB), which bloom in the spring and produce a fruiting crop in the summer, have been identified as short day (SD), once blooming, seasonal flowering (SF), seasonal berry (SB), single crop, or June-bearing plants (Cai *et al*., 2017). In

Table 1 - Shows the differences in runnering and cropping between Everbearing (EB) and Seasonal blooming (SB) strawberry varieties throughout the year

Type	Defined	Cropping over the year	Runnering	Commercially propagated
Everbearing (EB)	Remontant day-neutral (DN) perpetual LD plants	A couple of crops	Only non- to a few runners	Dividing of branch crowns
Seasonal flowering (SB)	once flowering seasonal flowering (SF) seasonal berry (SB) single cropping June-bearing short-day (SD)	One fruit crop	More runner	From the plantlets

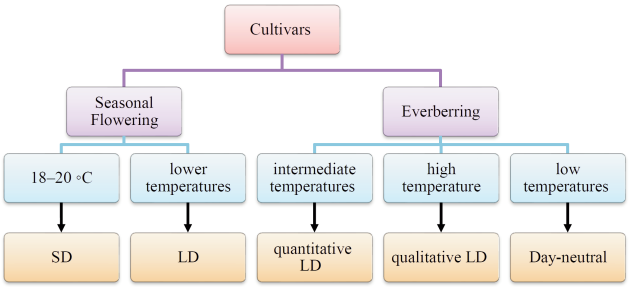

Fig. 2 - A schematic diagram shows the flower habit of strawberry cultivars that flower all year round is the key to propagation. Where: Everbearing (EB) behave as day-neutral (DN) plants at low temperature and are considered qualitative long-day (LD) at medium temperature and quantitative long-day (LD) at high temperature. Seasonal Flowering (SF) behave like long-day (LD) at lower temperature and like short-day (SD) at medium temperature.

response to the seasonally reducing photoperiod and temperature circumstances, SB strawberries begin to bloom in the late summer and fall, the year before blossoming and fruiting. The majority of SB cultivars are now regarded as facultative short-day (SD) plants as it has been established that they are mostly SD plants. At temperatures between 18 and 20 °C, they need SD for bloom induction, although at lower temperatures, the majority of cultivars begin flowering on long days (LD) (Ito and Saito, 1962; Heide, 1977; Heide *et al.*, 2013).

The crucial photoperiod for SD induction is 14–15 hours (Darrow and Waldo, 1934; Konsin *et al*., 2001) and the minimum number of SD cycles required for induction depends on the cultivar (Heide *et al*., 2013). The flower-inducing effect of SD, on the other hand, is temperature sensitive, peaking at intermediate temperatures and decreasing rapidly at temperatures above 21°C (Heide *et al*., 2013).

2. Propagation of strawberry

SB strawberry plants are commercially propagated from plantlets that multiply from the runner nodes of mature plants because technique is quicker than seed propagation and daughter plants retain the traits of their mother plant (Li *et al*., 2020). This plantlet is often created by nodes borne by runners or stolons that sprout from buds in the crown's leaf axils over the summer (Darrow, 1966). The runners often is elongated branch which have nodes and internodes running parallel to their length, with the bud at the first node usually being inactive (Ahmed and Ragab, 2003). It's length is due to the cell division and intermodal elongation in the plant are responsible for runner growth (Nishizawa and Hori, 1993). Therefore, one of the most important metrics is the number of runner and daughter plants produced by mature plants.

3. Environmental factors

The environmental conditions are one of the most crucial factors impacting the generation of strawberry runners. In all of the *Fragaria* genotypes examined, stolon formation and flowering induction can compete for space in the axillary meristems, and both developmental strategies are sensitive to environmental factors (Brown and Wareing, 1965; Guttridge, 1985; Bradford *et al*., 2010; Hytönen and Elomaa, 2011; Heide *et al*., 2013; Hytönen and Kurokura, 2020). In response to any alteration in the environment that encourages flowering (flowering habit), the strawberry produces a crown or stolon (Fig. 2). The photoperiod, chilling periods, and temperature are especially linked to these environmental or seasonal factors, and their interactions may have a major effect on strawberry dispersal (Andrés and Coupland, 2012; Salinas *et al*., 2017).

Photoperiod

Photoperiod is the duration of the daily exposure of an organism to illumination within hour (Cammack *et al*., 2008), it is defined as the period of time within a 24-hour time frame that light is available (Lanoue *et al.*, 2019).

One of the most crucial environmental factors for plants is light. Where, the plant is impacted by the length of the lighting period (photoperiod), the radiation strength, and the type of illumination wavelengths (colours). Plants employ photosynthesis, a process that uses light as an energy source, to produce secondary compounds and carbohydrates. Additionally, photoreceptors produce light that is utilised to detect and keep track of environmental changes (Chen *et al*., 2004). When it comes to the photoperiodic control of flowering in wild strawberries, phytochromes are crucial photoreceptors (Rantanen *et al*., 2014).

The photoreceptors' main module, the leaves, is capable of detecting a broad range of wavelengths, light intensities, and photoperiods. It controls the

essential gene proteins that the plant's developmental regulatory programme may use to transmit information about timing and light (Valverde, 2011; Shim *et al.*, 2016). Photoreceptors also enable plants to accurately monitor ambient light conditions and alter their development, morphology, and metabolic rates, including the start of blooming, in accordance with the particular environment in which they exist (Song *et al.*, 2018; Roeber *et al.*, 2022).

FLOWERING LOCUS T (FvFT1) and SUPPRESSOR OF THE OVEREXPRESSION OF CONSTANS1 (FvSOC1), two significant genes in the photoperiodic regulation of blooming and runners in woody strawberries, have provided some information on the photoperiodic control of FvTFL1 in seasonal flowering woodland strawberry. FvTFL1 integrates photoperiod and temperature signals to control flower induction, and higher FvFT1 mRNA levels are linked to earlier flowering under a variety of environmental conditions including light quality, photoperiod, and temperature, while turning off this gene significantly delays flowering (Hytönen and Kurokura, 2020).

The long-day photoperiod (LD) is one of the most important environmental elements affecting the growth and development of strawberries (Ito and Saito, 1962; Darrow, 1966; Heide, 1977; Okimura and Igarashi, 1997; Robert *et al.*, 1999; Heide and Sønsteby, 2007; Al-madhagi *et al.*, 2011; Hasan *et al.*, 2011; Li *et al.*, 2020). And in distinguishing strawberry runner axillary buds (Hytönen *et al*., 2009).

The effect of photoperiod on strawberry vegetative development and runner production has been widely discussed and has attracted the attention of numerous studies. Petioles length, leaf number, leaf area, and runner number and length all increase with LD photoperiod (Darrow, 1966; Sung, 1973; Plancher and Naumann, 1978; Nishizawa and Hori, 1993; Pipattanawong *et al.*, 1996; Robert *et al.*, 1999; Wiseman and Turnbull, 1999; Konsin *et al.*, 2002; Serçe and Hancock 2005; Sønsteby *et al.*, 2006; Hasan *et al.*, 2011; Li *et al.*, 2021 b). LD conditions promote cell division and cell elongation (Nishizawa, 1992; Nishizawa, 1994), due to an increase in the amount of endogenous gibberellins (GAs) that promote bud development in the plant (Taylor *et al*., 1994).

The number of hours during the LD photoperiod that the plant must be urged to produce a runner depends on a variety of factors, including cultivars and temperature. The photoperiodic cycle of 10 h light and 10 h dark failed to develop runners, but 14 h light and 14 h darkness did, and runner plants growing in LD were induced to flower provided they remained connected to parent plants growing in SD (Hartmann, 1947). As shown in figure 3, the impact of photoperiod (P) on strawberry propagation depends on a number of different parameters, including cultivar (C), cold storage (CS), temperature (T) and plant growth regulators (PGR).

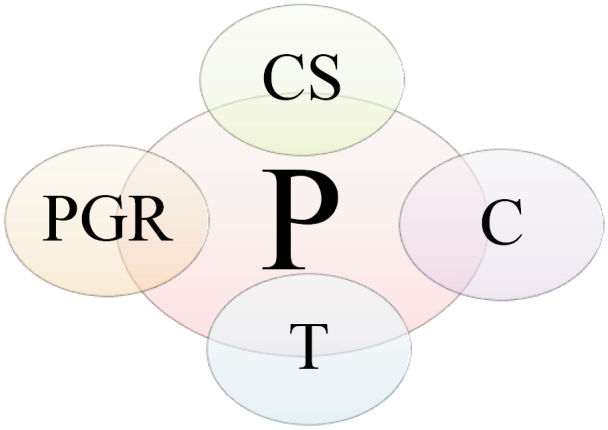

Fig. 3 - The response of strawberries to the photoperiod (P) effect on runner development is influenced by a number of other parameters, including cultivar (C), cold storage (CS), temperature (T), and plant growth regulators (PGR).

Photoperiod × cultivars (P×C)

The number and length of runners was influenced not only by photoperiod but also by cultivars or genetics, and the interaction of the two (Pipattanawong *et al.*, 1996; Serçe and Hancock 2005; Hasan *et al.*, 2011). In terms of runner production, the strawberry parents responded differently to the photoperiod.

The study by Serçe and Hancock (2005) shows the different responses of the wild strawberry genotype, *F. chiloensis* 'CFRA 0024' (Central Chile) and 'CFRA 0368' (Alaska) and *F. virginiana* 'Eagle 14' (Ontario); they just found that only 'Eagle 14' and 'CFRA 0368' produced an appreciable number of runners while 'Eagle 14' did not show a consistent trend, while 'CFRA 0368' had the most runners under the 11 hour photoperiod.

Clear photoperiod responses to vegetative development and stolon production were observed in SB cultivars (Plancher and Naumann, 1978; Konsin *et al.*, 2002; Sønsteby *et al.*, 2006; Hasan *et al.*, 2011), as well as on EB strawberry cultivars (Guttridge, 1969; Dennis *et al.*, 1970; Serçe and Hancock, 2005).

The difference between EB strawberry cultivars is

also evident: cultivars 'Aromas', 'Tribute', 'Frederick 9', and 'Fort Laramie' did not produce runners under either LD (16h) or SD (8 h), but 'Quinalt' produced 0.2 runner/plant under LD (16h) (Serçe and Hancock, 2005). Flower initiation and runner development occur independently of LD in EB strawberry cultivars (Piringer *et al*., 1958; Piringer and Borthwick, 1961; Guttridge, 1969; Dennis *et al*., 1970).

There was also a difference between SB cultivars, where 'Camaroga' yielded the most plantlets (22.06 per plant) when grown under the 17 hour photoperiod (Hasan *et al*., 2011).

According to Serçe and Hancock (2005), the LD photoperiod had a significant effect on runner production, with a significant effect of an interaction between the SB cultivar and the photoperiod, with 'Allstar' and 'Honeoye' not producing runners in photoperiod ranges of 8 -11 h, and 'Chandler' producing runners in photoperiod ranges of 8 , 9, or 11 h.

Strawberry SB developed more crowns than runners during the SD photoperiod compared to the plant under the LD (18 h) photoperiod on the 'korona' SB strawberry (Konsin *et al*., 2001). The number of runners is also differs between cultivars, in strawberry 'Seolhyang' it increased dramatically after LD (16 h) (Li *et al*., 2020). Meanwhile, for SB 'Camarosa' and 'Camaroga' cultivars, there was no significant difference between the 15 h and 17 h LD photoperiods, and the LD (15 h) was determined to be extremely efficient (Hasan *et al*., 2011).

Temperature (T)

Strawberry flowering habits (EB or SB) have been found to be under either qualitative or quantitative genetic influence (Heide *et al*., 2013). Figure 2 shows the behaviour of strawberries under different temperature conditions.

Wherever, the value of 10°C is a base temperature of strawberries (Al-madhagi *et al*., 2018). In *Fragaria vesca*, higher temperatures were found to be critical for runner induction (Heide and Sønsteby, 2007). The temperature variation between day and night at the same average daily temperature is also significant in creation runners; in strawberry 'Seolhyang', 25/15°C day/night was the best temperature for runners formation (Li *et al*., 2020).

LT (11°C) and SD (10 h) enhanced branch crown growth in wild strawberry *Fragaria vesca*, but HT (>18°C) promoted runner initiation independent of photoperiod (Bedry, 2017).

Regardless of temperature, all of the *F. x ananassa* EB cultivars showed very low runner counts. Temperature has an effect on runner formation in EB strawberries (Smeets, 1955; Smeets and Kronenberg, 1955; Serçe and Hancock, 2005). Controlling temperature alone, as well as cultivating plants at varied temperatures, will not increase EB strawberry runner output (Samad *et al*., 2021).

The EB cultivars responded differently to temperature in terms of runner production, with the greatest number of runner being 0.6 and 0.7 at 30°C in 'Aromas' and 'Tribute,' respectively. In contrast, below the T- range of 18-30°C, neither 'Ogallala' nor 'Quinalt' produced a runner (Serçe and Hancock, 2005).

A recent study Samad *et al*. (2021) found that runner production in EB strawberries decreased significantly at 20°C, with no statistical difference between 25 and 30°C. Runners was almost twice in EB strawberries 'Murano' than in 'Favori' during the season and it was significantly higher in the plants raised outdoors than in those raised in the greenhouse (Sønsteby *et al.*, 2022). The explanation for this is that EB's runner potential is cultivar dependant, and low temperatures (LT) put more energy into flowering than runner development (Rivero *et al.*, 2021 a; Sønsteby *et al.*, 2021).

Photoperiod × temperature (P×T)

Overall, the effect of photoperiod on runner and crown production was influenced by temperature at the time of photoperiod application. LD and high temperature (HT) have been shown to improve runnering in all flowering classes of strawberry cultivars (EB or SB) (Serçe and Hancock, 2005). When the photoperiod was 12 h or more and the temperature was above 10°C, runners began to multiply (Went, 1957; Darrow, 1966).

The strawberry's reaction to the photoperiod is affected by temperature (Darrow, 1936). Actual photoperiod and temperature parameters varied by cultivar (Went, 1957). The LD must exceed a certain value at HT for runner development in EB and SB strawberry cultivars (Darrow, 1937; Went, 1957; Smeets, 1980). Temperature and photoperiod promote runnering by inhibiting flower initiation and increasing the activation of vegetative buds on the rosette crown (Went, 1957; Leshem and Koller, 1965). Meanwhile, there was no runner development at SD LT, and LT at higher light intensities had to be suppressing flower initiation (Went, 1957). Strawberries can develop runners at a higher temper-

ature than at a lower temperature (Smeets, 1955; Smeets and Kronenberg, 1955; Went, 1957; Leshem and Koller, 1965; Smeets, 1980).

The ideal temperature for strawberries varies by cultivars. Under both LD and SD photoperiods, strawberry cultivars differ in the optimal temperature for runner development, and the number of runners produced increased as the temperature rose from 20 to 26°C under 16 h LD, but decreased as the temperature increased from 26 to 29°C (Bradford *et al*., 2010).

EB produced many runners during the 15 h - 20 h LD photoperiod with a temperature of at least 22.7°C (Darrow, 1966; Rivero *et al.*, 2021 a), no runners developed in 'Marshall,' at 10°C but did for 16 hours at 14°C, and for 12 hours at 17°C (Went, 1957). When the temperature dropped to 18°C, the LD photoperiod factor alone was sufficient for optimal leaf and inflorescence growth and development (Sønsteby *et al.*, 2006). Photoperiod preconditioned plants produced significantly more branch crowns than control plants, but cold-stored tray-conditioned plants produced much fewer crowns (Sønsteby *et al.*, 2006).

A LD of at least 14 hours was required for runner production in 9 cultivars cultivated at 13, 16, and 21°C in EB strawberry cultivars, where the photoperiod LD up to 14 h being the key determinant, a specific temperature being required for a prolonged runner development duration (Darrow, 1936). Flower initiation and runner formation in 'Revada' and 'Rabunda' occurred at 20 and 26°C regardless of the LD, and the length of runner formation was longer at 20 and 26°C than at 14°C, and at 16 and 24 h than at 8 h (Smeets, 1980).

Runners are formed almost entirely in the vegetative phase of plant growth in SB cultivars, with LD × HT favouring runner production (Darrow and Waldo, 1934; Heide, 1977; Durner *et al*., 1984; Bradford *et al*., 2010). SB cultivar also behaves like EB plants at LT under LD circumstances (14 h) (Darrow and Waldo, 1934; Darrow, 1936). The Honeoye SB cultivar did not develop runners at 14 or 17°C, regardless of photoperiod, and it did not produce runners under SD, independent of temperature (Bradford *et al*., 2010). In the number of runners of F1-hybrid 'Delizzimo' cultivar was significantly higher at 26°C than at lower 12°C under both SD and LD conditions (Samad *et al.*, 2022). Addition, higher temperatures increased the concentrations of sugars in the leaves in LD photoperiod (Rivero *et al.*, 2022).

The EB trait can also arise when inflorescences are removed during the growing season, leading to the development of latent buds, as in the LD and HT traits causing flowering suppression (Sugiyama *et al*., 2004). As shown in Table 2, the runner formation rises at HT × LD in both SB and EB.

Table 2 shows a rough summary of the influence of photoperiod and temperature interaction on the generation of runners. Despite the fact that the critical value of each variety is different, both groups agreed that the long day (LD) at high temperature is the best condition for the development of runners.

Chilling hour and cold storage (C)

Flower initiation in strawberries requires chilling (Ito and Saito, 1962; Darrow, 1966; Kinet *et al.*, 1993; Lieten, 1997; Al-madhagi *et al.*, 2018; Al-doubibi *et al.*, 2021). For the best production and berry quality, both types of strawberries (EB and SB) required different amounts of chilling period before planting. The chill-hours are measured in degrees below than 5, 7, or 8°C (Yanagi and Oda, 1993; Risser and Robert, 1993; Bigey, 2002; Gallace *et al.*, 2019). If the natural environment is not favourable, the refrigerator can be used to carry out cold treatments (Hamano *et al*., 2009). Chilling stimulates cell division and elongation by breaking dormancy (Lee *et al*., 1970; Yanagi and Oda, 1989).

The effect of chilling (up to zero and less than 5°C) or cold storage (below zero °C) on runner development has been connected to the type of strawberry (EB or SB), cultivars, degree of chilling, length of cold storage, and cumulative of natural chilling hours, according to the most recent study.

Longer cold treatments (more than 500 hours) limit flower development (Taghavi and Aghajani,

Table 2 - Interaction effect of photoperiod and temperature on runnering of strawberry

Factors		Cultivars	
Photoperiod	Temperature	EB	SB
SD*	Low	×	×
LD	Low	Some cultivars	×
DN	Low	×	×
SD	high	×	×
LD	high	√	√
DN	high	Some cultivars	√

*SD (short-day) is less than 14 hours, LD (long day) is more than 14 hours, and DN (day-neutral) is 12 hours. √: producing runner, ×: non-producing runner. Low= less than 20°C.

2017), lead to the shorter flower differentiation (Lieten, 2006; Al-madhagi *et al*., 2018) and delay the re-initiation of fresh floral primordial in the spring (Guttridge, 1958; Gallace *et al*., 2019). Strawberry propagation could benefit from this approach.

Chilling has been shown to increase runners generation in both EB and SB strawberry cultivars (Yanagi and Oda, 1990). Many runners were formed when the strawberry EB or SB cultivar was subjected to a lot of cooling hours (Bringhurst *et al*., 1960; Bailey and Rossi, 1965; Guttridge, 1969; Braun and Kender, 1985; Kahangi *et al*., 1992; Risser and Robert, 1993; Lieten, 1997; Tehranifar *et al*., 1998; Bigey, 2002; Hokanson *et al*., 2004; Taghavi and Aghajani, 2017; Al-madhagi *et al*., 2018).

The sensitivity of the chilling duration varies between cultivars; SB strawberry cultivars are more sensitive than EB cultivars, and prolonged chilling inhibits blossom production in SB cultivars (Yanagi and Oda, 1990).

After more than 1000 hours of chilling, EB cultivars formed runners (Hamano *et al*., 2009; Watanabe *et al*., 2009; Al-madhagi *et al*., 2018). Although the cultivar does not develop runners under normal conditions, and does not produce runners when chilled for 0 h, 360 h and 720 h, long chilling hours (1080 h and 1440 h) in a cold room at 2°C will reduce the flower and promote more runner (Al-madhagi *et al*., 2018). For Japanese EB strawberry cultivars ('Akihime', 'Askaruby', 'Sachinoka', 'Tochiotome', 'Toyonoka', 'Nyoho', and 'Yumenoka'), cold storage for more than 1000 hours interrupts dormancy, promotes runner development, and increases leaf elongation (Watanabe *et al*., 2009).

The duration of the cooling period for current EB strawberries is related to the cultivars. Chilling temperatures in EB cultivars start with runner development in 'Revada' and 'Rabunda' cultivars that have not experienced natural hours of chilling (Smeets, 1980), as well as in 'Rabunda', 'Ostara', and 'Kletter' cultivars refrigated at 1°C for 1 and 2 months (Yanagi and Oda, 1990).

Flowering degree and stolon production in EB strawberry cultivars Delizzimo and Favori had little or no effect when chilled at 2°C for six weeks (Rivero *et al.*, 2021 a). Furthermore, for a one to four weeks of chilling at 1°C increased runners in the cultivar EB 'Pajaro', with no significant difference in the length of cold storage, while one or two weeks of cold storage resulted in a larger number of daughter plants (Taghavi and Aghajani, 2017).

Longer cold storage duration improved runner production in the SB strawberry cultivars 'Hokowase' (Yanagi and Oda, 1990), 'Korona' and 'Elsanta' (Sønsteby and Heide, 2006), 'Allstar', 'Chandler', 'Latestar', 'Northeaster' and USDA selection B27' (Hokanson *et al*., 2004) and 'Sulhyang' (Lee *et al*., 2020).

SB strawberry cultivars stored chilled at 1°C for two months produced more runners than fresh plants that had never been exposed to cold (Hokanson *et al*., 2004). The degree of cold storage also influences runner quality. According to (Lee *et al*., 2020) Sulhyang' plants held at -5°C produced fewer daughter plants than those stored at -2°C, and the quantity of daughter plants was modest.

Long cold storage reduced vigour and glucose stores of mother plants (Lieten *et al*., 1995). Plants that have been stored cold for a long period should have a higher starch content and if possible, be cultivated in nurseries located at higher altitudes (López *et al*., 2002; Al-doubibi *et al*., 2021). On the other hand, naturally cool night-time temperatures at higher elevations help plants collect more starch. The quantity of chilling hours the plant experiences affects runner production; both insufficient chilling and excessive chilling have an effect (Hamano *et al*., 2009).

Photoperiod × cold storage (P × C)

As a result, exposure to prolonged photoperiods and longer cold storage duration improved runner production. The results of the previous study show that cultivars respond differently to photoperiod × cold storage and duration. After determining the cultivar type (EB or SB), this interaction is linked to the length of cold storage LC and LD photoperiod (Sønsteby and Heide, 2006; Hamano *et al.*, 2009; Watanabe *et al.*, 2009; Rivero *et al.*, 2021 a). Due to its insensitivity to the pre-chilling history and day duration, the EB strawberry 'Rabunda' showed consecutive flower development (Yanagi and Oda, 1989).

Meanwhile, more efficient runners production can be achieved in EB strawberry cultivars by combining cold storage with LD photoperiod, where LD (16 h) increase runner production by about 10% in plants chilled at 4°C that for 1000 and 1500 hours in comparison to unrefrigerated ones (Watanabe *et al*., 2009). The same result was observed in EB strawberry 'Natsuakari' and 'Dekoruju' treated with 1000 h chilling under 16 h LD (Hamano *et al*., 2009). EB strawberry cultivars 'Natsuakari' and 'Dekoruju'

chilled for 1500 and 2000 hours (5°C) produced runners above natural day length, but not below natural day length regardless of LD treatment.

In contrast, after 5 and 10 weeks of preconditioning at 2°C no runners occurred under either LD 10 h or 20 h, while runners were common in SD, particularly at 26°C and with 10 weeks of preconditioning (Rivero *et al.*, 2021 a).

Photoperiod enhanced the condition of *Fragaria* shoot cultures maintained at 4°C in SB strawberries (Reed, 2002). In SB strawberry cultivars 'Korona' and 'Elsanta', no cooling was required to re-establish normal leaf and inflorescence elongation and runner development under subsequent LD circumstances (Sønsteby and Heide, 2006).

4. Exogenous hormone

Gibberellins

Gibberellins are required for initiation of strawberry runners and inhibit GA production with PP333, AMO-1618, or prohexadione-calcium (Pro-Ca) (an inhibitor of the GA_3-oxidase enzyme) (Rademacher, 2000), causes the formation crown branches and reduces runner development (Avigdori-Avidov *et al.*, 1977; Nishizawa, 1993; Reekie and Hicklenton, 2002; Black, 2004; Hytönen *et al.*, 2009; Grez *et al.*, 2021). The GA20ox gene is mainly expressed in the axillary meristem dome and primordial, and in developing stolons. Runner less strawberries such as the woodland diploid strawberry (*F. vesca*) are caused by a mutation in the active site of a gibberellin 20-oxidase enzyme (GA20ox). As a result, GA3 stimulates runners development in all genotypes and species of strawberries, including the EB types of *F. vesca, F. virginiana*, and the EB and SB of *F. x ananassa* (Agafonov and Solovei, 1972; Solovei, 1972 a; Verzilov and Mikhteleva, 1974; Soetarto, 1979; Choma and Himelrick, 1984; Braun and Kender, 1985; Deyton *et al.*, 1991; Fouad *et al.*, 1991; Ra *et al.*, 1996; Dwivedi *et al.*, 1999 a, b; Paroussi *et al.*, 2002 a, b; Tenreira *et al.*, 2017; Li *et al.*, 2021 a; Godara *et al.*, 2022).

Overall, the effect of GA3 on runner growth was variable and dependent on GA concentration (Solovei, 1972 b; Mohammad *et al.*, 1990; Rajesh *et al.*, 2008), with GA3 at 50 ppm having no effect on runner growth in 'Sparkle' (SB) and 'Ozark Beauty' (EB) strawberries (Waithaka and Dana, 1978). According to Agafonov and Solovei (1974) GA3 administered to strawberries at a concentration of 0.005% improved the quantity of runners but decreased their quality. GA3 reduced runner growth at concentrations of 100 and 200 mg/L (Solovei, 1972 a). Application of 50 mg/L GA3 produced runner before flower in SD 'Camarosa' and 'Camroga' cultivars (Al-madhagi *et al.*, 2012). The number of strawberry 'Seolhyang' runners was reduced by GA3 foliar spray, which showed a negative correlation between the concentration and number of runners (Li *et al*., 2020). Effect of GA3 on runner growth varies between cultivar (Solovei, 1972 a; Choma and Himelrick, 1984), with GA3 stimulating daughter-plant formation in the EB cultivar but suppressing it in the SB cultivar (Waithaka and Dana, 1978; Choma and Himelrick, 1984). According to Kender *et al.,* (1971) the response of three EB cultivars to GA3 at 50 increased runner development in cultivars 'Ozark Beauty' and 'Superfection', but had no effect on cultivar 'Geneva'.

Due to longer internodes, EB strawberry 'Tribute' and 'Selva' cultivars treated with GA3 produced fewer daughter plants (Dale *et al*., 1996). Compared to NAA and CCC, GA3 produced the greatest vegetative growth and runner production at 90 ppm on 'Sweet Charlie' (Rajesh *et al*., 2008). GA3 use was related to the frequency of applied (Tafazoli and Vince-Prue, 1978; Duarte and Hermosa, 1998). GAs increased runner production when applied prior to the onset of dormancy and during the chill requirement stage (Honda, 1972), but did not increase the number of runners and hastened flowering when applied about a month before the appearance of flower buds, while hastened fruit maturation when applied at the flowers opening stage.

Cytokinins

Exogenous benzyladenine (BA) resulted in a greater numbers of runners in certain studies (Kour *et al*., 2017; Liu *et al*., 2019), while cytokinin and auxin coordinate the dormancy and expansion of axillary buds in strawberries (Qiu *et al*., 2019).

The influence of the exogenous hormone cytokinin on vegetative development has also been studied by several researchers, BA-type cytokinin has been observed by several researchers to enhance runner induction (Waithaka *et al*., 1978; Waithaka and Dana, 1978; Kour *et al*., 2017; Liu *et al*., 2019). 6-BA also enhanced runner induction, with 50 mg/L being the most effective concentration (Li *et al*., 2020).

In 'Sparkle' (SB) and 'Ozark Beauty' (EB) strawberries, foliar spraying with PBA at 200-600 ppm increased runner production (Waithaka and Dana, 1978).

In contrast, BA alone had no effect on the generation of runners such as EB cultivar 'Geneva' (Kender *et al*., 1971), SB cultivars 'Pajaro', 'Queen Eliza', and 'Paros' (Momenpour *et al*., 2011) and 'Redchief' (SB) (Archbold and Strang, 1986). PBA caused axillary bud explants to grow into stolons (Waithaka *et al*., 1980).

Interaction of exogenous hormone on runner development

The effect of the combining hormones on strawberry runner development is based on a fight between them that prevents flowering. In EB 'Geneva' the use of both N6B and GA3 had a significant impact on runner formation (Kender *et al*., 1971). In 'Ozark Beauty' (EB), a combination of PBA and GA3 had a stronger impact on runners and daughter plant development than PBA alone, and PBA reduced rooting of daughter plants, which GA3 could not overcome (Waithaka and Dana, 1978). When BA and GA3 were combined, petioles and stolon internodes were less thickened and elongated, resulting in greater leaf area than when PBA was used alone (Waithaka and Dana, 1978).

The number of runners in the EB 'Tribute' and 'Selva' strawberries treated with GA3 and BA increased linearly when the benzyladenine (BA) concentration was increased up to 1800 mg/L, the recommend that BA at 1200 mg/L + GA3 at 300 mg/L in strawberries, under field or greenhouse conditions for runner formation (Dale *et al*., 1996). Application of 6-BA + ACC resulted in the maximum number of plantlets (six plantlets per plant) (Kirschbaum, 1998). In EB, GA3 at 50 ppm, BA at 50 ppm, or companion boosted the number of runners in 'Miyoshi' by 2-3 fold, whilst GA3 or GA3 + BA raised the number of runners by up to 8 and 4 times in 'Enrai' and 'Summer Berry', respectively (Pipattanawong *et al*., 1996).

Gibberellic acid, when combined with benzyladenine, significantly increased runner development in the Geneva cultivar, but benzyladenine alone had little impact (Kender *et al*., 1971).

Photoperiod × PGR

Day length photoperiod and gibberellin alone both increase runner production in strawberry cultivars with different genotypes and blooming habits. Strawberry plant susceptibility to exogenous gibberellins was enhanced by LD photoperiods (Tafazoli and Vince-Prue, 1978; Al-madhagi, 2012). The LD photoperiod increased the level of endogenous gibberellins, which promoted the growth of plant buds (Taylor *et al*., 1994). Meanwhile, the LD photoperiod had the same effect as gibberellin, leading to a greater number of epidermal cells, indicating that cell division and internodes length were increased (Nishizawa and Hori, 1993; Nishizawa, 1994). Suppression of GAs biosynthesis has been shown to promote crown branching, restrict runner production, and improve flowering by increasing the number of possible sites for floral induction and differentiation (Hytönen and Elomaa, 2011; Tenreira *et al*., 2017).

In a prolonged photoperiod, exogenous GA3 completely reversed the effect of prohexadione-calcium when transferring GA3-treated plants from short to long days, on the other hand, it restored normal runner development, this did not happen in plants that had not been treated with GA3 (Hytönen *et al*., 2009)

The influence of photoperiod and exogenous hormone interaction on the vegetative development of strawberries has been documented mainly with GA3. After exposure to the LD photoperiod, GA3 elicited comparable change in strawberries (Paroussi *et al.*, 2002 a).

The study by Soetarto (1979) discovered that during the 24 h photoperiod GA3 at 150 ppm improved the stolon length of cultivar 'Ostara'.

SD, DN, and LD photoperiods plus GA3 (50 ppm) resulting in the greatest vegetative growth in the LD photoperiod with 50 ppm GA3 application and greatest number of crowns/plant when plants in the LD photoperiod and treated with 1000 ppm CCC (Dwivedi *et al.*, 1999 a). Plants grow faster when treated with GA3 in the LD photoperiod than in the SD photoperiod (Paroussi *et al.*, 2002 a).

By increasing the level of soluble sugar in 'Seolhyang' the strawberry cultivar, LD photoperiod (16 h) and 50 mg/L 6-BA break the dormancy of axillary buds and produced runners (Li *et al*., 2020).

Gibberellins can compensate for the effects of environmental variables

However, chilled strawberry plants treated with GA3 in tropical countries (Kenya) produced about the same number of runners as those subjected to chilling alone, but plants treated with BA produced significantly more runners than chilling alone (Kahangi *et*

al., 1992). When the plant was exposed to chilled conditions in conjunction with the treatment of BA + GA3, the number of runners increased (Kahangi *et al*., 1992). GA3 induced and enhanced vegetative growth, equivalent to the impact of four to six weeks of chilling (Tehranifar and Battey, 1997).

5. Discussion and Conclusions

The formation of strawberry runners was influenced by the interaction of genetic (cultivars), environmental (photoperiod, temperature, chilling hours or cold storage), and internal (hormones and carbohydrate) factors.

The most significant factor impacted by long-day photoperiod (LD) is the cultivar in strawberry runner proliferation. For the development of stolons in all flowering strawberry classes, LD and HT must interact. Additionally, in order for runners to grow in EB and SB strawberry cultivars, LD must surpass a certain value at HT (Darrow, 1937; Went, 1957; Smeets, 1980). This may have been connected to the influence of photoperiod on photosynthesis and the metabolism of carbohydrates, which suggested that the amount of carbohydrates may rise during the creation of runners and that the amount of soluble sugars was positively correlated with the number of runners (Li *et al*., 2020). Everbearing (EB) strawberries absorbed more CO_2 when temperature and irradiance rose (Rivero *et al*., 2021 b). However, the accumulation of photosynthates was not the only factor that affected the runner induction in cultivated strawberries (Li *et al.*, 2021 b). The photoperiod also enhanced the amount of endogenous hormone as well as the synthesis and accumulation of starch, sugar, amino acids, and protein (Li *et al.*, 2022). especially gibberellins that promote runner bud development (Taylor *et al*., 1994). And the effect of the application of gibberellins or photoperiod is the same result (Taylor *et al.*, 1994).

Meanwhile, more efficient runner production can be achieved in EB and SB strawberries by Gibberellins (GAs) Cytokinin and chilling period (CP) individually or in combination lead to production of stolons. In addition, CP or GAs enhances the effect of the photoperiod LD. Long cold storage or the chilling (CP) also works on converting starch to soluble sugars (López *et al.*, 2002; Al-madhagi *et al*., 2018; Al-doubibi *et al*., 2021), and increased level of endogenous gibberellins (Avigdori-Avidov *et al*., 1977).

In fact, greater photosynthesis and respiration under the LD condition imply that more chemicals and energy are produced, which may account for the increased soluble sugar content in strawberry seedlings during runner production (Li *et al*., 2020). In which the respiration produced ATP and hydrolyzed the sugar for biosynthesis, resulting in altered levels and ratios of endogenous hormones, maybe with a focus on gibberellins and cytokinin, by transferring more sugar to axillary buds that are in high demand while restricting the quantity of sugar via the apical shoot (Mason *et al.*, 2014). In strawberry runners (non-dormant buds), as opposed to dormant buds, the expression of genes involved in sugar metabolism and signalling was also increased (Qiu *et al*., 2019). In order to explain the changes in signalling between stages of bud release to sustained development, Cao *et al.* (2023) propose a model of apical dominance that combines auxin, sucrose, strigolactones, gibberellins, and cytokinin.

The application of cytokinin helped to break the apical dominance and shift the auxin/cytokinin ratio (Al-madhagi, 2012; Qiu *et al.*, 2019), which led to the growth of axillary buds to runner (Li *et al*., 2020). This cytokinin appears to promote runnering in early development stage, but prolonged, elevated it levels inhibit runnering.

The application of AB-6 increased the level of free active of endogenous gibberellins and auxin in strawberry seedlings to a value higher than the free active of endogenous cytokinin (Al-madhagi, 2012) and increased the soluble sugar (Al-madhagi, 2012; Li *et al*., 2020). This ultimately converted polysaccharides into soluble sugars and stimulated axillary buds to produce runners. Additionally, the photoperiodic control the two genes (FvFT1) and (FvSOC1), that regulation of blooming and runners of woody strawberries (Hytönen and Kurokura, 2020). This may be able to explain how the interaction of photoperiod and temperature influences the growth of runners by enhancing photosynthesis, elevating endogenous hormone levels, raising respiration, and raising the amount of soluble sugars. In order to fully develop a runner in SB strawberries or partially generate in EB strawberries, the plant makes advantage of the indirect effects of photoperiod or cold storage as shown in figure 4.

It can be concluded that, in all strawberry cultivars (EB or SB), soluble sugars may be required for axillary buds to emerge from their dormant case and produce runners, when this is impacted by the applica-

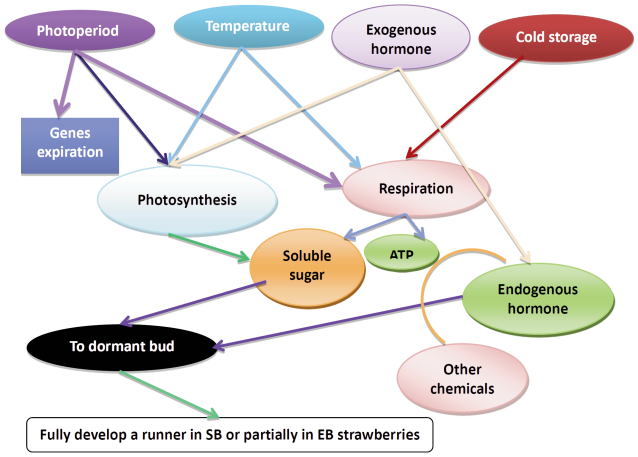

Fig. 4 - The effect of photoperiod, temperature, cold storage, and plant growth regulators alone or in combination prompted the plant to form runners by influencing the level of endogenous hormones and the level of sugar, whose level is raised in the axillary buds, prompting the plant to form runners.

tion of photoperiod LD or exogenous hormone.

The optimal strategy is determined by optimizing the planting date for propagation or changing the propagation conditions, depending on the cultivars and the seasonal environment (photoperiod × temperature) of the geographic region. Those factors are also important in the tissue culture technique as well as in the greenhouse or field.

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