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RESEARCH PAPER

Variation of the Leaf Area Index of Some Vegetables Commonly Grown in Greenhouse Conditions with Cultural Practices

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Abstract

Leaf area index (LAI) values in plants affect photosynthesis and carbohydrate production directly since it is a measure of photosynthetically active area and the area where transpiration occurs. Leaf area index is an important parameter required to determine plant water consumption by using climatic data and it is especially used in the calculation of aerodynamic resistance. Leaf area index varies depending on plant varieties and cultural practices and can be determined directly and indirectly by various methods. In this study, it was aimed to determine the LAI of four different crops (tomato, eggplant, cucumber and pepper) grown in Antalya, where greenhouse cultivation is intensive, depending on the cultural practices. The results showed that LAI was significantly affected by cultural practices such as leaf pruning and climatic differences. Leaf area index obtained from this study can be used to determine the crop evapotranspiration and aerodynamic resistance of four different plants grown under similar conditions.

1. Introduction

Leaf area index (LAI) is an important parameter needed in studies such as plant nutrition, competition between plants, soil-water relations, evapotranspiration, photosynthesis, plant protection measures, light reflection and heat transfer in crops (Karaca, 2020). Watson (1947) defined the LAI as the area of one-side green leaf tissue per unit area of land covered by the crop. Leaf area index determines the size of the crop-atmosphere interface, thus it plays a key role in the energy and mass exchange between the plant and the atmosphere (Weiss et al., 2004; Aydinsakir and Buyuktas, 2009). Changes in environmental conditions and differences in cultural practices affect leaf width and height, leaf number, yield and quality (Baudoin et al., 2013). In contrast, the ratio between leaf width and length is not affected by these differences (Rolland-Lagan et al., 2014).

Leaf area index varies according to the vegetation composition, stage of growth and seasons. In addition, it shows a significant variation due to the differences in growing conditions and cultural practices (Zhao et al., 2012). In greenhouse cultivation, cultural practices such as training, removal of new side shoots, shoot apices and leaves, leaf pruning, flower pruning and fruit pruning are made in order to achieve higher yield and quality per unit area (Tuzel, 2013). It is one of the most recommended agricultural practices in order to determine the number of the main stems that plants will continue to grow and to ensure that their development continues on these main branches, to increase yield and to make more use of sunlight for plants (Mendoza-Pérez et al., 2017).

There are various agricultural stereotyped cultural practices in greenhouse cultivation in Antalya. For example, in tomato plants, the leaves under the fruit that are big enough are plucked in order to make the fruit to color flushing faster. This application starts at the root collar of the plant and continues until it reaches the top of the plant. Therefore, very few leaves are left in the plant near

the removal of the plant (Ildır and Aktaş, 2018). Similar cultural practices are performed in the cucumber. The most important cultural practices affecting the LAI in eggplant and pepper are the number of branches left in the hang of string stage and leaf pruning. Therefore, the LAI varies regionally according to different cultural practices.

Greenhouse cultivation in Antalya are made on an area of 77 209 ha. Greenhouse production percentages can be expressed as 60% for tomato, 14% for cucumber, 11% for pepper, 4% for eggplant and 11% for others (TUİK, 2020). Due to the high contribution of greenhouse cultivation to the national economy, many academic studies are carried out. Leaf area index is needed especially in experiments related to crop water requirement (Karaca et al., 2018). In the FAO method ($ET_c = ET_o \times k_c$), which is widely used in evapotranspiration estimation, the regional calibration of the plant coefficient (k_c) is determined as a function of the LAI (Allen et al., 1998). For this reason, leaf pruning should be considered when determining the LAI. Morever, the LAI is also needed to determine aerodynamic resistance by dimensionless numbers (Reynolds (Re), Grashof (Gr), Nusselt (Nu), Rayleigh (Ra), Richardson (Ri), Prandtl (Pr), Schmidt (Sc), Sherwood (Sh), and Lewis (Le) (Stanghellini, 1987).

In this study, it was aimed to determine the seasonal variation of LAI values of vegetables (tomato, eggplant, pepper and cucumber) commonly grown under greenhouse conditions depending on the cultural practices in Antalya region.

2. Materials and Methods

This experiment was carried out for two growing seasons under the conditions of a lysimeter in a plastic greenhouse at Akdeniz University 30°38'30"N - 30°39'45"E, Antalya-Turkey. The first and second seasons of the experiment were conducted from September 14, 2018 to February 21, 2019 and from March 01, 2019 to July 15, 2019, respectively. Anıt F1 tomato, Ayda F1 cucumber, Corsica F1 eggplant and Buket F1 pepper varieties, which are suitable for both autumn and spring cultivation in Antalya Region, were used as plant material. The spacing between the rows, the spacing along the rows of plants and the number of plants in a parcel in the research are given in Table 1. These distances were chosen based on farmer behaviours.

Lysimeter soil had a silty-clay-loam texture (USDA, 1999) and irrigation water was in C_2S_1 (USSL, 1956) class. When 20% of the available soil moisture in the upper 60 cm soil profile was consumed, irrigation water was applied to bring the existing moisture to the field capacity. Leaf area (LA) was determined by a non-destructive method based on leaf dimensions (Karaca, 2020). The LAI of a plant canopy is defined as the one-sided green leaf area per unit ground surface area and is calculated as in Equation 1.

$$LAI=(LA\times n)/A \quad (1)$$

Where; LAI is the leaf area index ($cm^2\ cm^{-2}$); LA is the mean leaf area (cm^2); n is the number of leaves; and A is the area (cm^2).

All cultural practices were made taking into consideration the behaviour of the farmers. Leaf width (W), length (L) and the number of leaves for all plants were measured every 10 days during the growing period. The values on the days when these values were not measured were determined by interpolation method.

In the present study, the tomato was grown on a single central stem supported by a string when it reached 40 cm. The plant was checked regularly and new side shoots were removed from the plant. Furthermore, shoot apices were removed from the plant starting from the 8th fruit. Pepper and eggplant were trained to three main stems. These stems were supported by a string when it reached 40 cm. All leaves, branches and shoots under the selected stems were removed from the plant. The cucumber was cultivated on a single main stem and when it reached 30 cm in height, it was supported with a string. For better plant growth, all fruits and shoots up to 30 cm of plant height were removed from the plant. Leaf pruning was performed according to Ildır and Aktaş (2018) in tomato, MEGEP (2008b) in eggplant, MEGEP (2008a) in pepper and MEGEP (2007) in cucumber.

The soil was analyzed before planting for an accurate fertilization program. Fertilization was carried out by fertigation technique, considering the soil analysis and the growth of the plants. Crop water requirement was determined based on soil and irrigation water was given by drip irrigation

Table 1. The spacing between the rows, the spacing along the rows of plants and the number of plants in a lysimeter parcel in the research

Crop	Spacing between the rows (cm)	Spacing along the rows (cm)	Number of plants in a parcel (5 m^2)
Tomato	50	60	15
Eggplant	50	100	10
Pepper	50	60	15
Cucumber	50	60	15

system. The experiment was designed according to randomized block design with 3 replications.

3. Results and Discussion

Leaf are index for different growing seasons for tomato, eggplant, pepper and cucumber are given in Figures 1, 2, 3 and 4, respectively.

Leaf area index in tomato plants in both growing periods increased approximately until the 90th day after planting (DAP) (Figure 1). In the following days, it was determined that the LAI gradually decreased as a result of leaf pruning, which is one of the most common cultural practices in this region. The LAI of the tomato plant was approximately 1.5 $m^2 m^{-2}$ at the end of both growing periods while the highest LAI in the fall and spring periods was 4.1 and 4.3 $m^2 m^{-2}$, respectively. Harmanto et al. (2005) determined the LAI of cherry tomatoes under stress-free conditions stress-free conditions in greenhouse as 4.0 $m^2 m^{-2}$, while Al Mamun Hossain et al. (2017) obtained as 4.6 $m^2 m^{-2}$. Heuvelink et al. (2005) reported that the maximum LAI of tomato grown in the greenhouse was between 3.3 and 4.1 $m^2 m^{-2}$ and constantly changed during the season as a result of leaf pruning. Similarly, Ambroszczyk et al. (2008) stated that the LAI changed depending on the leaf pruning application.

The LAI of the eggplant plant increased continuously during the fall and spring periods and were determined as 10.9 and 7.7 $m^2 m^{-2}$ at the end of the growing periods, respectively (Figure 2). Tripathi et al. (2015) reported that the LAI of the eggplant irrigated with wastewater increased continuously until the 100th-day DAP and reached 4.2 $m^2 m^{-2}$, and then the crops did not grow. Yıldırım (2015) found that the LAI of the eggplant reached the highest level (2.8 $m^2 m^{-2}$) on the 88th of DAP under field conditions in a similar climate and it decreased continuously until the 113th of DAP (2.0 $m^2 m^{-2}$), which was the end of the growing season. Karam et al. (2011) announced that the maximum LAI of the eggplant varied approximately between 6.0 and 7.0 $m^2 m^{-2}$ during the two growing seasons and the LAI was constantly decreasing near the plant removal. Contrary to the studies in the literature, LAI of the eggplant showed an increasing trend in our study. Passioura and Angus (2010) declared that when vegetables were exposed to water stress, their life cycles shortened and physiological aging accelerated. Besides, since eggplant is a perennial plant, if the plant does not encounter stress, it continues to grow continuously. Therefore, a higher LAI was obtained in the fall period, when the growing period was longer than the spring period.

Similar to the eggplant, the LAI of the pepper increased continuously throughout the growing periods and reached 3.2 $m^2 m^{-2}$ in the fall period and 3.9 $m^2 m^{-2}$ in the spring period (Figure 3). Ta et al. (2011) reported that the LAI of pepper in the rock wool growing environment in the glasshouse increased continuously from seedling to plant removal and reached approximately 3.0 $m^2 m^{-2}$. Moreno et al. (2003) obtained the highest LAI (3.5 $m^2 m^{-2}$) from full irrigation in the study that different irrigation levels were applied in field conditions. On the other hand, Mendoza-Pérez et al. (2017) found that the number of the main stem in the plant also affected the LAI and the highest LAI (2.8 $m^2 m^{-2}$) was obtained from the plants growing on more than two main stems. Rubio et al. (2011) obtained the highest LAI (3.2 $m^2 m^{-2}$) of the pepper plant grown in different nutrient solutions at different salinity levels and depending on the number of main stems from the plant growing on three main stems. In the present study, the pepper plant was cultivated to be grown on three main stems, and the results obtained are consistent with the literature.

When the LAI of the cucumber in both growing seasons was examined, the LAI increased to 3.9 $m^2 m^{-2}$ until the 90th of DAP in the fall season, and 7.5 $m^2 m^{-2}$ until the 120th of DAP in the spring season (Figure 4). The differences in the fall and spring seasons were due to the greenhouse conditions and the cultural practices applied to the plant. In the region, leaf pruning in the fall period is more common than in the spring period. The most important reason for this is to protect the plant from fungal diseases caused by increased humidity and to create an airy root zone. Morever, the humidity inside the greenhouse increased significantly due to the closing of the roof ventilation on rainy days. In order to prevent this excessive moisture inside the greenhouse from damaging the plants, more leaf pruning was done in the fall seasons compared to the spring season. As a result, the LAI in the fall season was less than in the spring season. Al Mamun Hossain et al. (2017) stated that the maximum LAI of cucumber grown under conditions without stress was 3.0 $m^2 m^{-2}$ during the 70-day growing period. Nederhoff et al. (1988) announced that the LAI oscillated during the season and the maximum LAI was 3.5 $m^2 m^{-2}$. Similarly, in our study, it was determined that the LAI of cucumber went up and down in the fall season, while the maximum LAI was 3.9 $m^2 m^{-2}$. There are important differences between the LAI determined in the spring period and the LAI found in the literature. These differences were thought to occur due to the greenhouse conditions and the length of the growing period.

Yıldız (2018) determined the LAI of greenhouse plants in the Mediterranean region based on the plant growth stages [beginning (1), development (2), middle (3) and last (4) periods] using the method by Allen et al. (1998). The researcher reported that the LAI in the 3rd and 4th growth stages of the fall period was 3.2 and 2.9 $m^2 m^{-2}$ in tomato and 3.3 and 3.0 $m^2 m^{-2}$ in cucumber, respectively. In the spring season, the LAI of the crops in the 3rd and 4th development stages were 3.5 and 3.3 $m^2 m^{-2}$ in tomato; 3.2 and 3.0 $m^2 m^{-2}$ in eggplant; 3.1 and

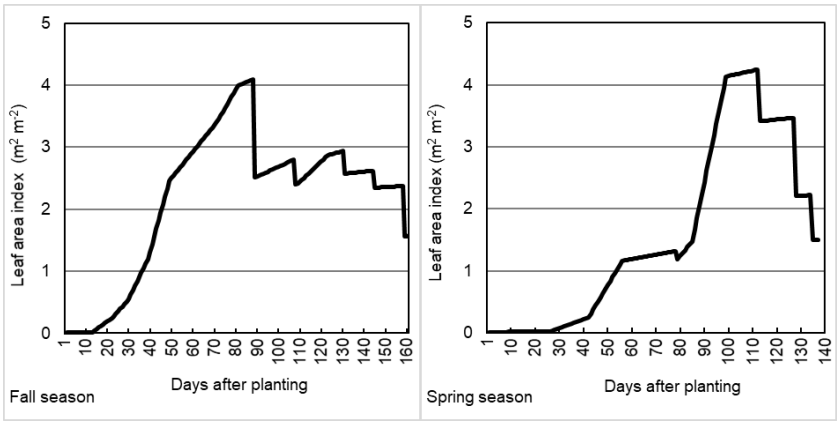

Figure 1. Seasonal change in leaf area index (LAI) for tomato

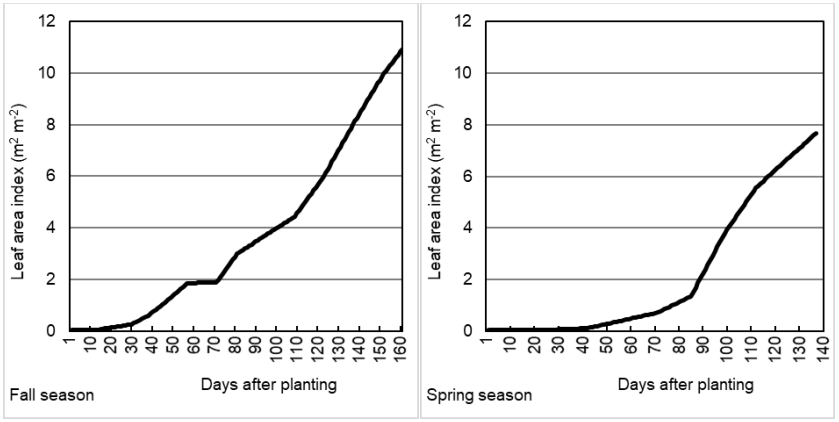

Figure 2. Seasonal change in leaf area index (LAI) for eggplant

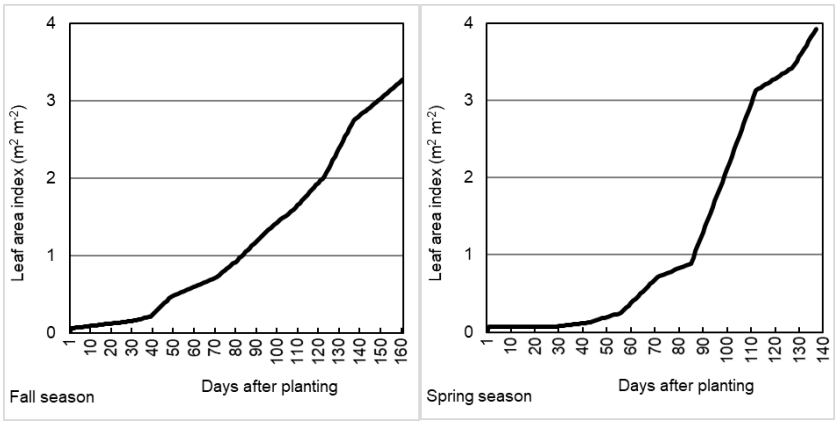

Figure 3. Seasonal change in leaf area index (LAI) for pepper

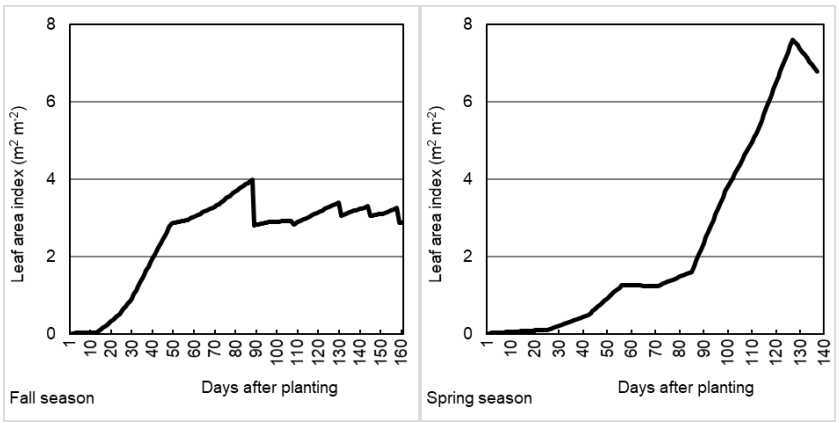

Figure 4. Seasonal change in leaf area index (LAI) for cucumber

3.0 m^2 m^{-2} in pepper; 3.6 and 3.4 m^2 m^{-2} in cucumber, respectively. When the LAI was examined, there were differences between our study and Yıldız (2018). One of the most important reasons for these differences was that Yıldız (2018) determined the LAI by assuming no leaf pruning practice. However, leaf pruning practice is widely carried out by the growers in the region. In addition, while in our study, models derived specifically for the region and plant varieties were used to determine the LA, Yıldız (2018) used models previously developed for other regions. Differences in the LAI occurred due to these reasons.

If we evaluate the seasonal changes of the LAI of all plants, in general, it was determined that the plant growth right after the planting seedlings at the beginning of the spring season was slower compared to the fall season. The reason for this was the low temperature in this period and the lower incoming solar energy compared to the other season. In addition, the highest LAI in all crops was observed in the spring season due to the suitable climate conditions. When the results obtained from this study and all the studies mentioned above were evaluated in general, it was determined that the LAI of the plants was affected by the greenhouse conditions and various cultural practices.

4. Conclusion

In this study, seasonal variation of the LAI of some vegetables commonly grown under greenhouse conditions were investigated. It was determined that the LAI was affected by cultural practices. Since leaf pruning is a common practice in greenhouse growing under Mediterranean conditions, this cultural practice should be taken into account in the methods developed to calculate the change of LAI. Otherwise, methods based on the LAI to calculate the crop coefficient will not be calculated correctly and therefore the crop evapotranspiration will be estimated incorrectly. Leaf area index obtained from this study can be used confidently in determining evapotranspiration and aerodynamic resistance under similar conditions.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Detection and Estimation of Genetic and Environmental Parameters through Model Fitting of Ten Bulb Yield Contributing Traits in Onion

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Abstract

Two onion varieties P_2 and P_3 and their products F_1 and F_2 were evaluated in summer and winter seasons for this investigation. Estimated mean values of different traits showed variations from generation to generation in each season. Values of six-parameters viz., $\hat{m}$, [d], [h], e_1, gd_1, gh_1 for all the characters were significant except gd_1 for a number of leaves, leaf length and bulb volume and also [d] for leaf length and neck length. Overall means '$\hat{m}$' had the highest magnitude than [d], [h], e_1, gd_1 and gh_1 for all the characters. Environmental parameter 'e_1' also exhibited higher magnitude than [d], gd_1 and gh_1. As the values of [d] and gd_1 were found to be non-significant, 4-parameter model was considered for leaf length only. Five-parameter model was considered for neck length, number of leaves and bulb volume and for rest of the traits 6-parameter model was considered. The goodness of fit test showed that 4, 5 and 6-parameter models were not adequate except bulb length and neck length. Therefore, for the development of these two traits in consideration of genotype × environment (G × E) interaction proper design and analysis needs to be done. Due to significant χ^2 values for other characters the situations becoming more complex as G × E interaction model is inadequate, so for their exact genetic explanation G × E model needs to be extended to include linkage and non-allelic parameters.

1. Introduction

Onion (*Allium cepa* L.) a member of the family Alliaceae is one of the most important spice crops grown all over the world. The use of onion is not limited to any climate or associated with nationality. It is popularly used both at immature and mature bulb stages as a vegetable and as a spice. Onion compared with other fresh vegetables, are relatively higher in food energy, intermediate in protein content, and rich in calcium and riboflavin. Onion has diuretic properties, beneficial to the digestive tract, good for the eyes, to act as a heart stimulant and useful as an anti-rheumatic remedy. It is a slow-growth, shallow-rooted crop with non-shading habitus and therefore its productivity is highly dependent on water availability in the soil, proper fertilization and weed control (Sekara et al., 2017).

As most commonly grown vegetable onion is on the list of 15, with respect to its importance, it has been provided the second rank following tomato and with respect to production, it takes the fourth rank in the world (Jahromi and Amirizadeh, 2015). Among the spice grown in Bangladesh, onion is grown in 172 460 ha and produced 1 802 868 metric tons (Mt) in terms of area and production during the year of 2018-2019 (BBS, 2019). Still, now Bangladesh is not sufficient in onion production though the per hectare yield and production increases but area decreases in the subsequent year (BBS, 2019). In this country, the average bulb yield of onion is 10 447 kg ha^{-1} (BBS, 2019). World dry bulb onion production increased 2.34 times between 1978 and 2002, whereas the population increased 1.45 times. The area under cultivation increased by a factor of 1.90 to 2.95 million ha in

this interval, and the world average yields increased from 14.04 to 17.40 t ha^{-1} (Brewster, 2008). Due to lack of quality seeds and improved varieties as well as improper cultural practices the yield level of onion is quite low (approximately 370-500 kg ha^{-1}) as compared to the higher yield (1000-1200 kg ha^{-1}) produced in other countries (Mila and Parvin, 2019). World production of onions and shallots (as green produce) was 4.5 million tons, led by China with 22% of the world total, and Japan, Mali and South Korea as secondary producers (FAO, 2019). Looking to the importance and production of this crop greater attention is needed for its improvement. Therefore, efforts should be made to develop high yielding varieties through breeding research. But the success of the breeding plan depends on the knowledge of genetic variability of population, about the nature and different gene actions governing the various quantitative traits. The breeder should able to determine in predicting the magnitude and extent of the effects of genotype × environment (G × E) interaction as an expression of genes, which are mostly related to environmental features.

The study of quantitative traits becomes complicated when more than one environment is included because changes in gene expression may occur with changes in environments. These changes, observable as G × E interaction in biometrical analysis, has long been recognized as an important source of phenotypic variation (Immer et al., 1934; Yates and Cochran, 1938; Mather, 1949). For specifying, estimation and correcting the effects of G × E interaction two main approaches have been used under regression.

The first one purely statistical analysis originally proposed by Yates and Cochran (1938) which was later on modified by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). The second approach is based on fitting of models which specifying the contribution of genetic and environmental effects and G × E interaction to generation mean and variance due to the contributions of additive, dominance and epistatic gene effects on the genetic and interaction components. This approach has been used by Mather (1949) and Jinks and Mather (1955), followed by Bucio Alanis (1966a), Bucio Alanis and Hill (1966b) and Perkins and Jinks (1968).

The study of G × E interaction in its biometrical aspects are important not only from genetic and an evolutionary point of view but also necessary to the agricultural production problem in general and particularly for plant breeding problems (Breese, 1969). Comstock and Moll (1963) reported that selection is impeded due to large effect of G × E interaction, knowledge about the description, prediction and inheritance of genotype interaction would provide more information and help the breeders to select better genotypes.

The breeding of adaptable onion varieties requires genotypes that have high stability for one or more quantitative traits. Information about adaptive potential and gene effects in onion are scanty for large scale exploitation inbreeding program. Although several information on genetical work in onion is available in the world but it is very few on G × E interaction following genetical approach based on first degree statistics.

In Bangladesh, no investigation on G × E interaction through weighted least square technique has been performed regarding onion. Therefore, the present investigation was undertaken to study G × E interaction on the basis of weighted least square technique for ten bulbs yield contributing traits of two onion varieties in two seasons to investigate the G × E interaction model is adequate or not.

2. Materials and Methods

The location of the experimental site is at 24º51ˈ N latitude and 89º22ˈ E longitude at an elevation of about 18 m from the average sea level. The experimental field was high land and non-calcareous grey / brown flood plain soils. The soil type was sandy to loam. Organic matter of the soil was 1.1 % with a pH value of 6.8 L is situated in Northern Bogra belonging to the Tista Meander Flood Plain which is under Agro-Ecological Zone (AEZ) number 3 (Anonymus, 1988).

The study was conducted at the central farm of Spices Research Center (SRC), Bangladesh Agricultural Research Institute (BARI), Shibgonj, Bogra, Bangladesh. Seeds were sown on May 05 and seedlings were transplanted on June 15, 2005.

Two released onion varieties such as BARI Piaz-2 (P_2) and BARI Piaz-3 (P_3), their product F_1 (P_2 × P_3) and F_2 produced in two seasons viz., summer (S) and winter (W) of the year 2005, were the materials in this study. Twenty cross combinations for F_1 (including reciprocals) bulb production and twenty for F_2 (including reciprocals) bulb production as well as 5 parents (produced by selfing) of onion were considered as 45 treatments in this trial.

The experiment was set up in a randomized complete block design with three replications. The size of each plot was 3.0 × 1.0 m. The space between row and plant was 15 × 10 cm. The treatments were distributed at random within each of the blocks.

Selfing was done by putting individual bamboo-made frame with cotton net (20 mesh) over the plants as soon as the first flower opened. Then flies were introduced to ensure pollination. Besides, after anthesis the umbels were rubbed against each other daily for a few days to ensure self-pollination. This rather inexpensive method of selfing is used when only a small quantity of seeds is needed (Jones and Mann, 1963).

Data on ten characters viz., bulb diameter, bulb length, neck diameter, neck length, plant height, number of leaves, leaf length, bulb weight, bulb

volume and bulb yield /plot were taken from 20 and 25 randomly selected plants for F_1 and F_2, respectively. Collected data were analysed through the standard biometrical techniques in the following sub-heads.

2.1. Detection and estimation of genetic and environmental parameters

The approach based on fitting models, the specification of the environmental contribution to the phenotypes depending on the experimental design was given by Mather and Jones (1958). It was further extended by Bucio Alanis and Hill (1966b) and Bucio Alanis et al., (1969). Following them, the phenotypic values in a particular environment of the following generation may be written as:

$$P_{ij} = \hat{m} + [d] + e_j + gd_j$$
$$P_{2j} = \hat{m} - [d] + e_j - gd_j$$
$$F_{1j} = \hat{m} + [h] + e_j + gh_j$$
$$F_{2j} = \hat{m} + {}^1/_2\,[h] + e_j + {}^1/_2\,gh_j$$

The model was fitted consisting of $\hat{m}$, [d], [h], e_1, gd_1 and gh_1 by weighted least squares and testing its goodness of fit using chi-square (χ^2) for 2, 3 and 4 df (df= number of generations – number of parameters used). Among the parents and seasons, P_3 and winter season were arbitrary and considered as increasing, and those P_2 and the summer season was considered as decreasing. The six-parameter G × E interaction model is given Table 1.

2.2. Estimation of the mean values and standard errors

Mean: Data on individual plant basis were added together then divided by the total number of observations and the mean was obtained as follows:

$$Mean\ (\overline{X}) = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where, X_i = individual reading recorded from each plant, $\sum X_i$ = total number of observations, n = number of observations, i = 1, 2, 3,….., n and $\sum$= summation.

Standard error of mean (SE): If several samples are taken, the standard deviations of different samples will vary. These variations are measured by the standard error as follows:

$$SE = \sqrt{\frac{S^2}{n}}$$

Where, S_2= variance and n= number of observations.

2.3. Estimation of $\hat{m}$, [d], [h], e, gd_1 and gh_1 and their standard errors

Estimation of $\hat{m}$, [d], [h], e, gd_1 and gh_1 and their standard errors by using their co-efficient were calculated as follows:

$$\hat{m} = \frac{1}{8}(1 \times \overline{P_3} + 1 \times \overline{P_3} + 1 \times \overline{P_2} + 1 \times \overline{P_2} + 1 \times \overline{F_1} + 1 \times \overline{F_1} + 1 \times \overline{F_2} + 1 \times \overline{F_2})$$

$$[d] = \frac{1}{8}\left(0 + 0 + 0 + 0 + 1 \times \overline{F_1} + 1 \times \overline{F_1} + 1 \times {}^1/_2\,\overline{F_2} + 1 \times {}^1/_2\,\overline{F_2}\right)$$

$$[h] = \frac{1}{8}(1 \times \overline{P_3} + 1 \times \overline{P_3} - 1 \times \overline{P_2} - 1 \times \overline{P_2} + 0 + 0 + 0 + 0)$$

$$e = \frac{1}{8}(1 \times \overline{P_3} - 1 \times \overline{P_3} + 1 \times \overline{P_2} - 1 \times \overline{P_2} + 1 \times \overline{F_1} - 1 \times \overline{F_1} + 1 \times \overline{F_2} - 1 \times \overline{F_2})$$

$$gd_1 = \frac{1}{8}(1 \times \overline{P_3} - 1 \times \overline{P_3} - 1 \times \overline{P_2} + 1 \times \overline{P_2} + 0 + 0 + 0 + 0)$$

$$gh_1 = \frac{1}{8}\left(0 + 0 + 0 + 0 + 1 \times \overline{F_1} - 1 \times \overline{F_1} + 1 \times {}^1/_2\,\overline{F_2} - 1 \times {}^1/_2\,\overline{F_2}\right)$$

SE of $\hat{m}$, [d], [h] ,e, gd_1 and gh_1

$$= \sqrt{\frac{1}{64}[(SEP3)^2 + (SEP3)^2 + (SEP2)^2 + (SEP2)^2 + (SEP2)^2 + (SEF1)^2 + (SEF1)^2 + (SEF2)^2 + (SEF2)^2]}$$

Table 1. The six-parameter G × E interaction model

Generation	Season	Mean	Variance	W_i= 1/variance	Full Model					
					$\hat{m}$	[d]	[h]	e_1	gd_1	gh_1
P_3	W				1	1	0	1	1	0
P_3	S				1	1	0	–1	–1	0
P_2	W				1	–1	0	1	–1	0
P_2	S				1	–1	0	–1	1	0
F_1	W				1	0	1	1	0	1
F_1	S				1	0	1	–1	0	–1
F_2	W				1	0	½	1	0	½
F_2	S				1	0	½	–1	0	-½

Where, W= winter season, S= summer season, Wi= weight, $\hat{m}$= mid parent value, [d]= additive effects, [h]= dominance effects, e1= differences between two environments, gd1= measures the interaction between additive and environmental components, and gh1= measures the interaction between dominance and environmental components.

Table 2. The parameters of the goodness of fit

Generation	Season	Observed (O_i)	Expected (E_i)	$(O_i - E_i)^2$	W_i	$\chi^2 = (O_i - E_i)^2 \times W_i$
P_3	W					
P_3	S					
P_2	W					
P_2	S					
F_1	W					
F_1	S					
F_2	W					
F_2	S					
						$\chi^2 = \sum(O_i - E_i)^2 \times W_i$

Where, W = winter season, S = summer season, Wi= weight

2.4. Estimation of expected mean value

The expected mean value of all generations derived from the estimated values of $\hat{m}$, [d], [h], e_1, gd_1 and gh_1 were calculated as follows:

$$M = J^{-1} \times S$$

Where, M= estimate of the parameters, J= information matrix, J^{-1}= inverse of the information matrix and S= matrix of scores.

After perform the matrix, the expected mean of all generations are as follows:

$$\overline{P_3}\, in\ W = \hat{m} + [d] + e_1 + gd_1$$

$$\overline{P_3}\, in\ S = \hat{m} + [d] - e_1 - gd_1$$

$$\overline{P_2}\, in\ W = \hat{m} - [d] + e_1 - gd_1$$

$$\overline{P_2}\, in\ S = \hat{m} - [d] - e_1 + gd_1$$

$$\overline{F_1}\, in\ W = \hat{m} + [h] + e_1 + gh_1$$

$$\overline{F_1}\, in\ S = \hat{m} + [h] - e_1 - gh_1$$

$$\overline{F_2}\, in\ W = \hat{m} + {}^1/_2\,[h] + e_1 + {}^1/_2\, gh_1$$

$$\overline{F_2}\, in\ S = \hat{m} + {}^1/_2\,[h] - e_1 - {}^1/_2\, gh_1$$

In case of 4-parameter model for leaf length excluding [d] and gd_1 analysis was done. Regarding 5-parameter model for neck length excluding [d] and for number of leaves and bulb volume excluding gd_1 analyses were done.

2.5. Testing the goodness of fit using in 4, 5 and 6-parameter G × E interaction models

The goodness of fit was tested by using the Table 2. Where the calculated χ^2 values were compared with 2, 3 and 4 df depends on how many parameters used in the model. If the χ^2 value is significant, it indicates that the G × E interaction model is inadequate and the estimate of the model is biased to an unknown extent. A failure of this model may be attributed to one or more reasons given below (Singh and Pawer, 2005):

(a) The presence of epistasis, that is, the adequacy of the specification of genetic contribution,

(b) Unjustified reduction in the number of environmental parameters, that is, incomplete specification of the environmental contribution, and

(c) The presence of G × E interaction.

3. Results and Discussion

The simple additive-dominance model assumes that gene differences contribute independently from one another to variation in the phenotype. The additive-dominance model further assumes that gene differences and environmental differences also contribute independently of one another to variation in the phenotype. We must turn to consider the interaction of gene and environmental differences, how much interaction may arise and how it can be detected, measured and investigated. For the estimation of genotype × environment (G × E) interaction in the experiment different seasons in different years and locations are needed. Environmental differences arise due to

heterogeneity of the environment to which the individuals are distributed. This leads to the difference between both segregating and non-segregating individuals grown in the same experiment. Specification of the environmental contribution to the phenotype depends on the experimental design, this in turn determines the specification of G × E interaction.

Mean with standard error in different generations of each variety in two seasons were different for all the ten quantitative characters are presented in Table 3. The mean values show variations from generation to generation in both two varieties and seasons for each of the characters. The maximum mean values for all the characters were obtained for all the generations in winter season. The highest mean was observed in F_1 generation in winter season for all the characters. Parent P_3 in winter season performed better compared to F_2 with the maximum values of means for all the characters except bulb length. Similar trend was also observed regarding summer season. Comparatively the lowest mean values were recorded in F_2 generation in summer season for most of the characters.

The six-parameter $\hat{m}$, [d], [h], e_1, gd_1, gh_1 and their standard errors were estimated and their significant tests of each of the parameters for all the ten quantitative characters were done separately and are presented in Table 4. Table 4 shows that the values of each parameters for all the characters are significant except gd_1 for number of leaves, leaf length and bulb volume and also [d] for neck length and leaf length. Significant $\hat{m}$, [d], [h], e_1, gd_1 and gh_1 values for bulb diameter, bulb length, neck diameter, plant height, bulb weight and bulb yield/plot indicated the presence of additive and dominance effects and also G × E interaction. Non-significant value of gd_1 for number of leaves, leaf length and bulb volume indicated the absence of additive × environment interaction. Similar analysis in two varieties of *Nicotiana rustica* was done by Bucio Alanis (1966a) and reported that there was no evidence of G × E interaction as gd_1 found to be non-significant when compared with standard error although there were significant additive genetic [d] and environmental (e_1) effects noted for final plant height. Bucio Alanis (1966a) analyzed the data mean final height of two inbred lines P_1 and P_2 of *N. rustica* from the results of an experiment initiated in 1946 by Professor Mather and his colleagues. The experiment was conducted at the John Innes Institute in London from 1946 to 1948, and from 1950 to 1964 at the University of Birmingham and observed that two inbred lines show different responses to the changing environment, although an interpretation of the nature of the different responses (G × E) is not obvious. Bucio Alanis (1966a) also concluded from generation mean analysis using the same data that genotype × environmental interaction is linearly related to the environmental effect. On the basis of the G × E interaction analysis Bucio Alanis (1966a) defining the best genotype as having (a) the highest performance over environments and (b) the highest stability of performance (lowest variance over the possible environments). Overall means 'm' had the highest magnitude than [d], [h], e_1, gd_1 and gh_1 for all the characters in this investigation. Dominance effect [h] was also higher in magnitude than other parameters regarding all the characters. Environmental effect e_1 also exhibited higher magnitude than [d], gd_1 and gh_1. The values of additive effect [d] for leaf length and neck length were found to be non-significant although gd_1 was significant for neck length. On the other hand, there was no evidence of additive × environment interaction 'gd_1' for number of leaves, leaf length and bulb volume. The significant values of gd_1 indicated the evidence for additive × environment interaction as well as significant values of gh_1 indicated the presence of dominant × environment interaction.

As the values of [d] and gd_1 were found to be non-significant, so, 4-parameter model consisting of $\hat{m}$, [h], e_1, and gh_1 was considered for leaf length only (Table 5). Five-parameter model consisting of $\hat{m}$, [d], [h], e_1, and gh_1 was considered for number of leaves and bulb volume (Table 5). Another 5-parameter model consisting of $\hat{m}$, [h], e_1, gd_1 and gh_1 was used for neck length (Table 5). Six-parameter model (Table 5) consisting of $\hat{m}$, [d], [h], e_1, gd_1 and gh_1 was considered for the rest of six traits as all the parameters were found to be significant for these characters.

Chi-square (χ^2) testing of goodness of fit of model including four-parameter for one character, five-parameter for three characters and six-parameter for the rest six quantitative characters with two varieties of onion in two seasons were done separately and are shown in Table 5. This table showed that all of the four, five and six-parameter models were not adequate as indicated by their significant $\chi^2_{(4)}$, $\chi^2_{(3)}$ and $\chi^2_{(2)}$ values for all the characters except for neck length and bulb length. Similar trend of results in two and three parameters models were found by Azad (1991) in lentil. Researcher also reported that in case of 4-parameter model, the non-significant χ^2 values for all the six characters indicated the adequacy of G × E interaction model. Genetical approach of G × E interaction model based on first degree statistics was also explained by Mather and Jones (1958) and gave specifications of various phenotypes in terms of biometrical genetic parameters. Bucio Alanis (1966a) developed a biometrical genetic model to explain the G × E interaction and applied this model to *Nicotiana rustica* data on two inbred lines grown at two different locations over 16 years and observed the linear relationship between the environmental effect and G × E interaction. Bucio Alanis and Hill (1966b) extended of Bucio Alanis (1966a) model to include heterozygote and applied it to *N. rustica* data and again observed the similar result of Bucio Alanis

Table 3. Mean values with standard error (SE) and their weight (W_i) of four generations of ten bulb yield contributing traits in onion

Generations	Season	Mean ± SE	W_i	Mean ± SE	W_i
		Bulb diameter (cm)		Bulb length (cm)	
P_3	W	4.3067 ± 0.0287	20.2225	4.8783 ± 0.0233	30.7031
P_3	S	3.5333 ± 0.0172	56.0224	4.6333 ± 0.0234	30.5158
P_2	W	4.1450 ± 0.0227	32.2685	5.0133 ± 0.0251	26.4620
P_2	S	3.0333 ± 0.0169	58.2072	5.0667 ± 0.0229	31.837
F_1	W	4.7117 ± 0.0293	19.3949	6.0033 ± 0.0273	22.3564
F_1	S	3.4333 ± 0.0207	38.9864	5.4167 ± 0.0347	13.8408
F_2	W	3.9867 ± 0.0340	11.5500	5.0133 ± 0.1139	1.0280
F_2	S	2.6367 ± 0.0412	9.8348	4.3667 ± 0.0550	5.5072
		Neck diameter (cm)		Neck length (cm)	
P_3	W	0.8367 ± 0.0100	164.2036	1.8667 ± 0.0171	57.1102
P_3	S	0.7333 ± 0.0116	124.6883	1.4500 ± 0.0140	85.5432
P_2	W	0.9117 ± 0.0109	139.8601	1.7200± 0.0109	64.3915
P_2	S	0.5700 ± 0.0073	265.2520	1.5183 ± 0.0191	45.7457
F_1	W	1.1083 ± 0.0139	86.0585	2.000 ± 0.0165	61.4628
F_1	S	0.8633 ± 0.0110	137.3626	1.6000± 0.0249	26.8168
F_2	W	0.8987 ± 0.0194	15.4108	1.2080 ± 0.0351	10.8260
F_2	S	0.7667 ± 0.0153	70.8215	1.5667 ± 0.0572	5.1245
		Plant height (cm)		Number of leaves	
P_3	W	47.2167 ± 0.0792	2.6596	5.8333 ± 0.1041	1.5391
P_3	S	35.5000 ± 0.0770	2.8095	5.700 ± 0.1017	1.6121
P_2	W	42.3333 ± 0.0614	4.4250	5.6667 ± 0.0812	2.5286
P_2	S	31.8833 ± 0.1065	1.4683	5.1500 ± 0.0884	2.1338
F_1	W	47.4667 ± 0.0805	2.5727	6.3000 ± 0.1147	1.2661
F_1	S	35.8667 ± 0.1268	1.0363	5.8000 ± 0.0974	1.7560
F_2	W	42.3733 ± 0.3071	0.1413	5.6400 ± 0.1180	0.9576
F_2	S	30.2667 ± 0.4522	0.0813	5.0000 ± 0.1188	1.1800
		Leaf length (cm)		Bulb weight (gm)	
P_3	W	36.3333 ± 0.1227	1.1063	30.6667 ± 0.0999	1.6698
P_3	S	23.6333 ± 0.1188	1.1816	20.1667 ± 0.1191	1.3308
P_2	W	35.5000 ± 0.0905	2.0345	29.2167 ± 0.1092	1.3987
P_2	S	19.9167 ± 0.1172	1.2144	18.4000 ± 0.1145	1.2716
F_1	W	37.3333 ± 0.1133	1.2107	31.0833 ± 0.1122	1.3234
F_1	S	25.3333 ± 0.1132	1.3015	20.7500 ± 0.1026	1.5839
F_2	W	33.8533 ± 0.3875	0.0888	27.9733 ± 0.3013	0.1468
F_2	S	24.0333 ± 0.2820	0.2063	18.3333 ± 0.2466	0.2774
		Bulb yield plot^{-1}		Bulb volume (cm^3)	
P_3	W	7.6333 ± 0.0263	24.0500	17.0000 ± 0.1438	1.6110
P_3	S	5.0333 ± 0.0344	14.0706	14.6667 ± 0.3333	0.6000
P_2	W	7.3400 ± 0.0289	19.9045	16.3333 ± 0.1541	1.4030
P_2	S	4.2660 ± 0.0251	26.4201	13.400 ± 0.2350	1.2069
F_1	W	7.7100 ± 0.0271	22.5648	21.3333 ± 0.1465	1.5536
F_1	S	5.2133 ± 0.0351	13.5648	15.6667 ± 0.2108	1.5000
F_2	W	6.9733 ± 0.0390	8.7819	16.0000 ± 0.4180	0.1908
F_2	S	4.6267 ± 0.0612	4.4571	13.6667 ± 0.3737	0.4773

W = winter season, S = summer season

Table 4. Estimated values of $\hat{m}$, [d], [h], e, gd_1 and gh_1 and their standard error from 6-parameter model of ten bulb yield contributing traits in onion

Characters	$\hat{m}$	[d]	[h]	e_1	gd_1	gh_1	Standard error
Bulb diameter	3.7233*	0.0827*	1.4321*	0.5642*	-0.0423*	0.2442*	0.0097
Bulb length	5.0490*	-0.0710*	2.0138*	0.1782*	0.0373*	0.1137*	0.0178
Neck diameter	0.8361*	0.0110*	0.3505*	0.1028*	-0.0298*	0.0389*	0.0046
Neck length	1.6162*	0.0098^{NS}	0.6234*	0.0825*	0.0269*	0.0276*	0.0101
Plant height	39.1133*	1.0625*	14.9567*	5.7342*	0.15833*	2.2066*	0.0740
Number of leaves	5.6363*	0.0896*	2.1775*	0.2238*	-0.0479^{NS}	0.1025*	0.0367
Leaf length	29.4921*	0.0544^{NS}	1.2061*	0.7749*	0.0487^{NS}	0.4082*	0.0693
Bulb weight	24.5738*	0.4021*	9.3733*	5.1613*	0.8359*	1.8942*	0.0591
Bulb yield plot^{-1}	6.0996*	0.1325*	2.3404*	1.3146*	0.7072*	0.4588*	0.0128
Bulb volume	16.0083*	0.2417*	6.4792*	1.6583*	-0.075^{NS}	0.8542*	0.0961

* and NS indicate significant and non-signifiant, respectively.

Table 5. Chi-square (χ2) values following 6, 5, and 4-parameter models of ten bulb yield contributing traits in onion

Bulb diameter (cm)

Generations	Mean	$\hat{m}$	d	h	e	gd	gh	Expected mean	Wi	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	4.3067	1	1	0	1	1	0	4.2788	20.2225	0.0008	0.0158
P_3	3.5333	1	1	0	-1	-1	0	3.4933	56.0224	0.0016	0.0894
P_2	4.1450	1	-1	0	1	-1	0	4.1275	32.2685	0.0003	0.0099
P_2	3.0333	1	-1	0	-1	1	0	2.9948	58.2072	0.0015	0.0864
F_1	4.7117	1	0	1	1	0	1	4.5896	19.3949	0.0149	0.2890
F_1	3.4333	1	0	1	-1	0	-1	3.3501	38.9864	0.0069	0.2701
F_2	3.9867	1	0	½	1	0	½	4.3964	11.5500	0.1678	1.9384
F_2	2.6367	1	0	½	-1	0	-½	3.2971	9.8348	0.4361	4.2887
											$\sum\chi^2_{(2)} = 6.9876^{*}$

Bulb length (cm)

Generations	Mean	$\hat{m}$	d	h	e	gd	gh	Expected mean	Wi	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	4.8783	1	1	0	1	1	0	4.8746	30.7031	1.4255	0.0004
P_3	4.6333	1	1	0	-1	-1	0	4.6025	30.5157	0.00095	0.0291
P_2	5.0133	1	-1	0	1	-1	0	5.0089	26.4620	1.9279	0.0005
P_2	5.0667	1	-1	0	-1	1	0	5.0372	31.8370	0.00087	0.0277
F_1	6.0033	1	0	1	1	0	1	5.9929	22.3564	0.00011	0.0024
F_1	5.4167	1	0	1	-1	0	-1	5.2807	13.8408	0.0185	0.2558
F_2	5.0133	1	0	½	1	0	½	5.4673	1.0280	0.2061	0.2119
F_2	4.3667	1	0	½	-1	0	-½	5.0503	5.5072	0.4673	2.5735
											$\sum\chi^2_{(2)} = 3.1013^{NS}$

Neck diameter (cm)

Generations	Mean	$\hat{m}$	d	h	e	gd	gh	Expected mean	Wi	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	0.8367	1	1	0	1	1	0	0.5543	164.2036	0.0797	13.0882
P_3	0.7333	1	1	0	-1	-1	0	1.5665	124.6883	0.6942	86.5628
P_2	0.9117	1	-1	0	1	-1	0	0.5628	139.8601	0.1217	17.0230
P_2	0.5700	1	-1	0	-1	1	0	0.9735	265.252	0.1628	43.1862
F_1	1.1083	1	0	1	1	0	1	1.1205	86.05852	0.0001	0.0128
F_1	0.8633	1	0	1	-1	0	-1	0.7949	137.3626	0.0047	0.6431
F_2	0.8987	1	0	½	1	0	½	0.8395	35.4107	0.0035	0.1238
F_2	0.7667	1	0	½	-1	0	-½	1.0325	70.8215	0.0706	5.0033
											$\sum\chi^2_{(2)} = 165.6432^{***}$

Plant height (cm)

Generations	Mean	$\hat{m}$	d	h	e	gd	gh	Expected mean	Wi	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	47.2167	1	1	0	1	1	0	48.7240	2.6596	2.2719	6.0424
P_3	35.5000	1	1	0	-1	-1	0	45.7121	2.8095	104.287	292.9973
P_2	42.3333	1	-1	0	1	-1	0	43.5870	4.4249	1.5718	6.9553
P_2	31.8833	1	-1	0	-1	1	0	42.5989	1.4683	114.824	168.5940
F_1	47.4667	1	0	1	1	0	1	47.3257	2.5727	0.0199	0.0512
F_1	35.8667	1	0	1	-1	0	-1	42.9163	1.0363	49.6971	51.5011
F_2	42.3733	1	0	½	1	0	½	46.7406	0.1413	19.0732	2.6958
F_2	30.2667	1	0	½	-1	0	-½	43.5359	0.0815	176.073	14.3534
											$\sum\chi^2_{(2)} = 543.1905^{***}$

Bulb weight (g)

Generations	Mean	$\hat{m}$	d	h	e	gd	gh	Expected mean	W_i	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	30.6667	1	1	0	1	1	0	30.4644	1.6698	0.0409	0.0683
P_3	20.1667	1	1	0	-1	-1	0	20.2394	1.3308	0.0053	0.0070
P_2	29.2167	1	-1	0	1	-1	0	29.5815	1.3987	0.1331	0.1862
P_2	18.4000	1	-1	0	-1	1	0	17.8863	1.2716	0.2639	0.3356
F_1	31.0833	1	0	1	1	0	1	31.1234	1.3234	0.0016	0.0021
F_1	20.7500	1	0	1	-1	0	-1	20.5051	1.5839	0.0600	0.0950
F_2	27.9733	1	0	½	1	0	½	30.5732	0.1468	6.7595	0.9923
F_2	18.3333	1	0	½	-1	0	-½	25.2640	0.2740	48.0351	13.1616
											$\sum\chi^2_{(2)} = 14.8481^{***}$

Bulb yield plot^{-1}

Generations	Mean	$\hat{m}$	d	h	e	gd	gh	Expected mean	W_i	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	7.6333	1	1	0	1	1	0	8.6115	24.0500	0.9567	23.0096
P_3	5.0333	1	1	0	-1	-1	0	4.9182	14.0710	0.0132	0.1864
P_2	7.3400	1	-1	0	1	-1	0	2.9013	19.9045	19.7024	392.1654
P_2	4.2666	1	-1	0	-1	1	0	0.3045	26.4201	15.6986	414.7591
F_1	7.7100	1	0	1	1	0	1	7.7524	22.5648	0.0018	0.0407
F_1	5.2133	1	0	1	-1	0	-1	5.3217	13.5648	0.0117	0.1592
F_2	6.9733	1	0	½	1	0	½	6.7544	8.7819	0.0479	0.4209
F_2	4.6267	1	0	½	-1	0	-½	3.9665	4.4571	0.4358	1.9424
											$\sum\chi^2_{(2)} = 832.6837^{***}$

Table 5. Chi-square (χ2) values following 6, 5, and 4-parameter models of ten bulb yield contributing traits in onion (cont.)

Neck length (cm)

Generations	Mean	$\hat{m}$	h	e	gd	gh	Expected mean	W_i	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	1.8667	1	0	1	1	0	1.8174	57.1102	0.0024	0.1387
P_3	1.4500	1	0	-1	-1	0	1.4317	85.5432	0.0003	0.0285
P_2	1.7200	1	0	1	-1	0	1.7115	64.3915	0.0001	0.0047
P_2	1.5183	1	0	-1	1	0	1.5376	45.7457	0.0004	0.0170
F_1	2.0000	1	1	1	0	1	1.9431	61.4628	0.0032	0.1991
F_1	1.6000	1	1	-1	0	-1	1.6023	26.8168	0.0000	0.0001
F_2	1.2080	1	½	1	0	½	1.8538	10.8260	0.4170	4.5145
F_2	1.5667	1	½	-1	0	-½	1.5435	5.1245	0.0005	0.0028
										$\sum\chi^2_{(3)} = 4.9054^{NS}$

Number of leaves

Generations	Mean	$\hat{m}$	d	h	e	gh	Expected mean	W_i	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	5.8333	1	1	0	1	0	-11.9707	1.5391	316.9833	487.8690
P_3	5.7000	1	1	0	-1	0	23.1158	1.6121	303.3110	488.9677
P_2	5.6667	1	-1	0	1	0	-11.9695	2.5286	311.0364	786.4868
P_2	5.1500	1	-1	0	-1	0	23.1170	2.1338	322.8129	688.8182
F_1	6.3000	1	0	1	1	1	-7.5799	1.2661	192.6514	243.9159
F_1	5.8000	1	0	1	-1	-1	19.4376	1.756	185.9853	326.5902
F_2	5.6400	1	0	½	1	½	-9.7750	0.9576	237.6222	227.5471
F_2	5.0000	1	0	½	-1	-½	21.2770	1.1800	264.9416	312.6311
										$\sum\chi^2_{(3)} = 3562.8260^{***}$

Bulb volume (cm^3)

Generations	Mean	$\hat{m}$	d	h	e	gh	Expected mean	W_i	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	17.0000	1	1	0	1	0	16.3607	1.6111	0.4087	0.6584
P_3	14.6667	1	1	0	-1	0	16.4803	0.600	3.2892	1.9735
P_2	16.3330	1	-1	0	1	0	14.7641	1.4032	2.4616	3.4542
P_2	13.4000	1	-1	0	-1	0	14.8836	1.2069	2.2012	2.6566
F_1	21.3333	1	0	1	1	1	21.1874	1.5536	0.0213	0.0331
F_1	15.6670	1	0	1	-1	-1	15.3707	1.5000	0.0876	0.1314
F_2	16.0000	1	0	½	1	½	18.3749	0.1908	5.6402	1.0761
F_2	13.6667	1	0	½	-1	-½	15.5264	0.4773	3.4583	1.6506
										$\sum\chi^2_{(3)} = 11.6339^{**}$

Leaf length (cm)

Generations	Mean	$\hat{m}$	h	e	gh	Expected mean	W_i	$(O_i - E_i)^2$	$W_i \times (O_i - E_i)^2$
P_3	36.3333	1	0	1	0	27.74277	1.1063	73.7973	81.6419
P_3	23.6333	1	0	-1	0	31.40086	1.1816	60.3345	71.2913
P_2	35.5000	1	0	1	0	27.74277	2.0345	60.1747	122.4253
P_2	19.9167	1	0	-1	0	31.40086	1.2144	131.8866	160.1631
F_1	37.3333	1	1	1	1	37.12479	1.2107	0.0435	0.0526
F_1	25.3333	1	1	-1	-1	25.13640	1.3015	0.0388	0.0505
F_2	33.8533	1	½	1	½	32.43378	0.0888	2.0151	0.1789
F_2	24.0333	1	½	-1	-½	28.26863	0.2063	17.9378	3.7006
									$\sum\chi^2_{(4)} = 439.5042^{***}$

*, **, ***, and NS indicate significant at 5%, 1%, 0.1% level, and non-significant, respectively.

(1966a) that found earlier. Bucio Alanis et al. (1969) further extended this G × E interaction model to include F_2 and backcross generations in the analysis and predicted the relationship between potence, heterosis and additive environmental effects (Singh and Pawar, 2005). To determine the stability and adaptability performance of onion, statistical approach of G × E interaction model was also performed by Golani et al. (2005), Jokanovic et al. (2016), and Tahir et al. (2020). Results of the present investigation shows that out of the ten characters only for neck length and bulb length with the genetic and environmental effects, G × E interaction effect is also present due to adequate of G × E interaction model. So, in the future breeding experiments for the development of these two traits proper design and analysis needs to be done for consideration of G × E interaction. However, in other characters due to significant χ^2 values the situation becoming more complex as G × E interaction model is inadequate to explain the genetic nature of these traits and hence for their genetic explanation need more generations as well as need to extend the G × E interaction model including other parameters like non-allelic interaction and linkage either individually or both at a time.

4. Conclusions

It is now recognized that G × E interaction is an important source of phenotypic variations. As under the control of gene, breeders are trying to produce and select suitable cultivars, which gave maximum economic yield over a range of environments with wider adaptabilities and stabilities. In the breeding program usually many potential genotypes are

evaluated in different environments before selecting certain desirable traits. In the present investigation, chi-square values for all the characters except bulb length and neck length are found to be significant which reveal that except additive genetic, dominance genetic and G × E interaction effects the other genetical effects may present in these traits that's why need to enlarge the G × E interaction model including linkage and non-allelic parameters either individually or both for getting the exact genetic information of these bulb yield contributing traits as well as stable onion genotypes over all the agro climatic regions.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Effects of Chilling Injury, Physical and Biochemical Changes on Grafted Watermelons Stored at Low Temperature

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Abstract

Watermelon fruit from Crimson Tide (CT) and Crisby (CR) grafted onto Ferro, RS841, Argentario, and Macis rootstocks and ungrafted CT and CR were compared for effects of low temperature storage on chilling injury, physical and biochemical changes at 0°C and 85–90% relative humidity for 21 days. After storage, fruit were hold to 21°C and 75–80% relative humidity for 7 days to determine shelf life. Quality analyses were determined during storage and shelf life at a weekly interval. The chilling injury areas covered <25% of rind surface of fruit for both cultivars. Weight loss in grafted and control fruit were very low (<1%) during storage for both cultivars. Fungal decay was not observed during storage for both cultivars, but it was seen during the shelf life for both cultivars. Total soluble solid content remained above 10% in fruit of both cultivars throughout storage period. Lycopene content significantly decreased at the end of storage for both cultivars.

1. Introduction

Soil borne diseases (caused by *Fusarium* and *Verticillium* species etc.) due to continuous and intensive cultivation are limiting factors affecting early season watermelon cultivation in plastic tunnels and later in open field conditions. *Lagenaria* and *Cucurbita* rootstocks are known to be resistant to Fusarium wilt and other soil-borne diseases; it provides advantages to watermelon cultivation to control diseases, to eliminate plant rotation and to increase yield as an alternative to other disinfection methods. The primary reason for grafting watermelon has been for *Fusarium* resistance, but it can be used to provide resistance or increase tolerance to Phytophthora blight, Verticillium wilt, Phomopsis rot, root-knot nematodes and in some cases viruses (Davis et al., 2008). Grafting has also been effective at increasing the cold tolerance of watermelon (Miguel et al., 2004). In addition, grafting impress fruit quality such as flesh firmness, fruit pulp color, lycopene and sugar amount. There is little work on the postharvest physiology of grafted or ungrafted watermelons. Storage and shelf of watermelons is confined by low temperature (<7°C) and high temperature. Fruits are susceptible to chilling injury and flesh color rotting and loss of color at lower temperatures and fruit are exposing to rotting and sugar loss at higher temperatures (Chisholm and Picha, 1986). The usual shelf life for watermelon is 14–21 days after harvest at 13°C (Rushing et al., 2001). Watermelons are generally not cooled when shipped locally. But, watermelons ripen in the hot summer months and are exposed to high temperatures during marketing. Cold storage and shipping can be preferred during export shipping to extend shelf life.

In the Mediterranean basin, where agricultural land for long rotations is unavailable, use of resistant rootstocks, largely interspecific cucurbit hybrids has become imperative for watermelon production (Kyriacou and Soteriou, 2015). Reports

on watermelon quality with respect to grafting have been conflicting, indicative of a rootstock-dependant effect or a rootstock-scion interaction (Yetisir et al., 2003; Miguel et al., 2004; Davis and Perkins-Veazie, 2005; Taylor et al., 2006).

Postharvest quality of watermelon fruit from Crisby (CR) and Crimson Tide (CT) grafted onto Ferro, RS841, Argentario and Macis rootstocks and non-grafted CR and CT were determined in 21 days during the storage at 7°C. The storage period of watermelons in good quality were determined as 21 days at 7°C. In both cultivars, watermelons grafted on Ferro and RS841 rootstocks preserved their postharvest quality better than non-grafted fruits and other rootstocks (Özdemir et al., 2016, 2018).

The aim of this study was to carry out the effects of low temperature storage on chilling injury and other quality criteria's of watermelon fruit from Crimson Tide and Crisby grafted onto Ferro, RS841, Argentario, and Macis rootstocks during storage at 0°C and 90±5% relative humidity for 21 days and shelf life at 21±0.5ºC and 70±5% relative humidity for 7 days compared to fruit from ungrafted Crimson Tide and Crisby.

2. Materials and Methods

2.1. Materials

The experiment was carried out at the Republic of Turkey Ministry of Agriculture and Forestry Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. The watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cultivars Crimson Tide (CT) and Crisby (CR) were grafted onto Ferro and RS841 (*Cucurbita maxima* x *Cucurbita moschata*) and Argentario and Macis (*Lagenaria siceraria*) rootstocks by using slunt-cut grafting method (Lee and Oda, 2003). The grafted plants were supplied by the commercial seedling company of Grow Fide (Antalya, Turkey). The non-grafted CT and CR were used as control.

2.2. Physical, biochemical changes and chilling injury

Fruits were harvested at full maturity when the 75% of tendril and stipule on the same node with peduncle were desiccated. After harvest, fruit were stored at 0±0.5C and 90±5% relative humidity for 21 days in cold store and hold 21 days at 0°C and subsequent 7 days at 21±0.5ºC and 70±5% relative humidity for shelf life.

Changes in weight loss (%), the incidence of fungal decay (%), fruit flesh firmness (N), total soluble solids (%), juice pH, titratable acidity (%), ripening (1–7), citric and malic acid (%), lycopene (µg g^{-1}), β-carotene (µg g^{-1}), hallow heart (1–5), fructose (%), glucose (%), sucrose (%), total sugar (%), sensory quality (1–9), flesh colour (L*, C* and hº) values, chilling injury in rind (external) and flesh (internal) (1–5) were determined during storage and shelf life at a weekly interval.

Weight loss (%); 30 fruit were numbered and the weight loss was determined in reference to initial weight of fruit with a laboratory balance sensitive until 0.01 g for grafted and ungrafted fruit of both cultivars. Fruit flesh firmness (N); the heart portion of the fruit was measured with a penetrometer (Now FHR-5 Nippon Optical Works Co. Ltd. Tokyo, Japan) having a drilling head at a conical probe of 12-mm in the force in kilograms and the results were translated into newton (N). Total soluble solid (TSS) content (%); TSS content was determined on juice obtained from 5 watermelons per replicate with the help of a handheld refractometer (Atago Model ATC-1E Atago Co. Ltd., Tokyo, Japan) at 20°C and calculated in percent. juice pH; pH was measured by digital pH-meter (Orion 5-Star model Thermo Fisher Scientific Inc., MA, ABD). Titratable acidity (TA) content (%); TA content was measured by employing potentiometric method for measuring TA content, 5 ml of fruit juice obtained from 5 watermelons per replicate were completed until 100 ml and titrated with 0.1 N NaOH to pH 8.1 (expressed as g malic acid per 100 mL juice). The fruits were also scored at each evaluation for chilling injury (CI) in the rind (external) and flesh (internal) and decay (1 = none, 2 = <10% of surface area, 3 = 11% to 25%, 4= 26% to 50%, and 5 = > 50%) (Risse et al., 1990). Incidence of CI and decay were determined after 7, 14 or 21 days at 0°C and 7 days at 21°C following each storage duration. Sensory quality (1–9) of fruit was rated with 1–9 hedonic scale. 1: very bad and 9: the best values show on this scale, hallow heart (1–5) of fruit were rated on hedonic scale of 1=none to 5=very severe (50% "more than hallow heart) and ripening (1–7) of fruit were rated on hedonic scale of 1= raw fruit and 3=mature to 7=over-ripe extremely by trained ten panelists. Fruit flesh color was assessed as reflected in the CIELAB (L*a*b*) color space using a CR-300 Minolta Chroma Meter (Konica Minolta, Osaka, Japan), calibrated using the manufacturer's standard white plate. Twice reading was made from the flesh of fruit. Flesh color L* value indicates lightness, ranging from 100 (white) to 0 (black). Flesh color Chroma (C*) value indicates color saturation, which varies from matt (poor value) to vivid color (top value) and it was computed using the formula (a2 + b2)1/2. Flesh color Hue angle (h°) value defines an angle from a color wheel with red-purple (0°), yellow (90°), bluish green (180°), and blue (270°), and it was determined by h°=tan−1 (b/a), (McGuire, 1992).

Sugars and organic acids analysis were performed in homogenized frozen watermelon samples. For this process, the samples were slipped through Whatman No. 4 filter paper under vacuum and 20 µL of watermelon sample was syringed immediately into the HPLC (LC-10A Series, Shimadzu Co., Kyoto, Japan) equipment. The HPLC analysis of sugars were performed on

equipment consisting of a refractive index detector and Nucleosil Carbohydrate column (250 mm × 4 mm, Macherey-Nagel, Düren, Germany) with 2 mL min^{-1} flow rate at 25°C, all of the sugars analysis was at 210 nm. The HPLC analysis were determined on equipment including of a photodiode array detector of organic acids and a TransgenomicTM ICSep ION 300 column (300 mm × 7.8 mm, Transgenomic San Jose, CA, USA) with 0.4 mL min^{-1} flow rate at 65°C, all of the organic acids analysis was at 210 nm (Chisholm and Picha, 1986). For sugar analysis of the mobile phase was comprised of acetonitrile and water at 2 mL min^{-1} flow rate. Acetonitrile: distilled water (80:20, v/v) mixture was used as a mobile phase. For organic acids analysis of the mobile phase was consisted 0.0085 N H_2SO_4 at 0.4 mL min^{-1} flow rate. The results were related to g 100 g^{-1} fresh weight.

Analysis of carotenoids was performed in homogenized frozen watermelon samples. For this process, t the homogenization of the frozen watermelon samples were done using a 10 mm shaft and a low speed of Ultra-Turrax homogenizer. The purees (3 g) were taken into the centrifuge tube and obtained with HPLC-grade solvents of 5 mL of ethanol, 5 mL of acetone and 10 mL of hexane including 0.05% butylated hydroxytoluene (Merck KGaA) and 20 µL of the sample, which was the top hexane layer was filtrated with a 0.45-µm Millex-HV filter (Millipore), was syringed instantly into Shimadzu HPLC equipment (LC-10A Series, Shimadzu Co., Kyoto, Japan). The HPLC analysis of carotenoids were performed on equipment consisting of a photodiode array detector and a YMC carotenoid C30 column (250 mm × 4.6 mm, YMC Europe GMBH) with 1.5 mL min^{-1} flow rate at 30 °C, the lycopene analysis was at 503 nm and the β-carotene analysis was at 452 nm (Perkins-Veazie and Collins 2006). For carotenoids analysis of the mobile phase was comprised of methyl tertiary butyl ether, methanol and deionized distilled water (15:81:41, solvent A), methyl tertiary butyl ether and methanol (90:10, solvent B) (Liu et al., 2009). The results were defined as µg g^{-1} fresh weight.

2.3. Statistical analysis

The study was carried out during a 2-year period and data are expressed as the mean of 2 experimental years. The data were analysed a completely randomized block design by ANOVA using SAS software of SAS Institute, Cary, N.C. (SAS, 2019). The data were obtained from three replicates per scion/rootstock combination. Each replicates contained 5 fruit. The mean separation at *P <0.05* level was made with Fisher's Least Significance Test.

3. Results and Discussion

Weight loss in grafted and control fruit were very low (<1%) during storage for both cultivars. In CT cultivar, fruit on RS841 rootstock resulted in higher weight loss than those on other rootstocks and control fruit at the end of the storage time and control fruit resulted in higher weight loss than those on other rootstocks at the end of the storage time and shelf life. In CR cultivar, fruit on RS841 rootstock and control fruit resulted in higher weight loss than those on other rootstocks at the end of the storage time and fruit on RS841 rootstock and control fruit resulted in higher weight loss than those on other rootstocks during the storage time and shelf life periods (Figure 1, 2, 3 and 4). Consistent with our results, Perkins-Veazie and Collins (2006) and Özdemir et al. (2016, 2018) reported the <1% of weight loss in watermelon fruit during storage or shelf life. However, Araújo Neto et al. (2000) determined higher weight loss (3.8%) than our results. Suárez-Hernández et al. (2016) reported that some rootstocks caused to reduce in weight loss during storage periods.

Fungal decay was not observed during storage for both cultivars but, except during the shelf life. The decayed areas covered <10% of rind surface of fruit. The graft combinations did not differ in the incidence of fungal during shelf life for both cultivars (Figure 5 and 6). Fungal decay that occurred during shelf life after storage at 0°C might be due to

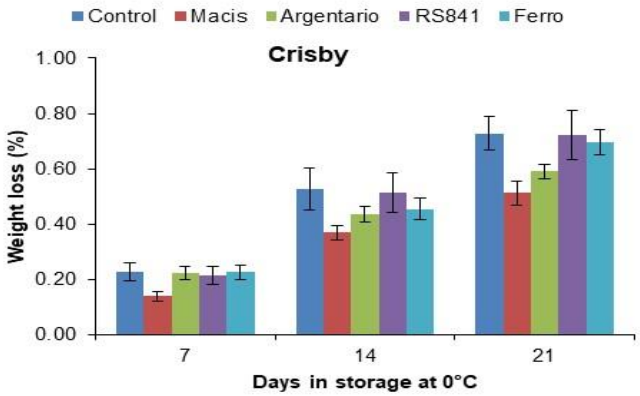

Figure 1. The effects of rootstocks on weight loss of Crisby watermelon fruits during storage

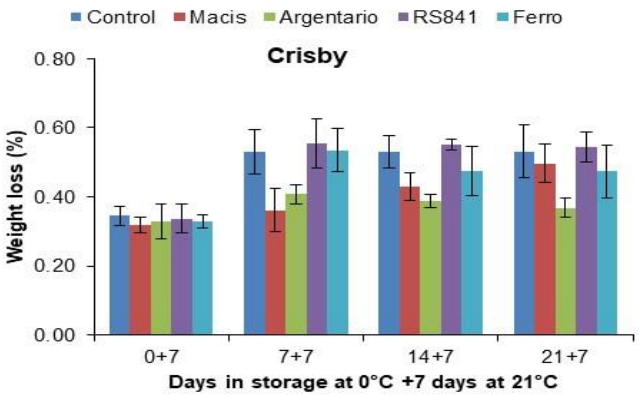

Figure 2. The effects of rootstocks on weight loss of Crisby watermelon fruits during shelf life

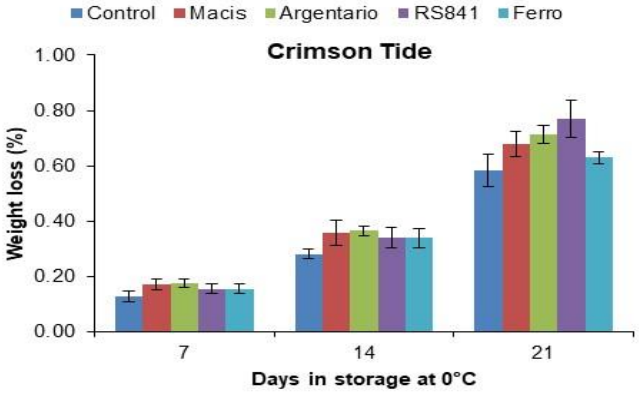

Figure 3. The effects of rootstocks on weight loss of Crimson Tide watermelon fruits during storage

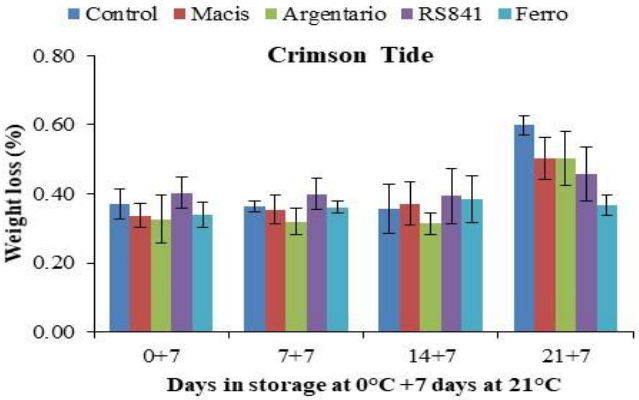

Figure 4. The effects of rootstocks on weight loss of Crimson Tide watermelon fruits during shelf life

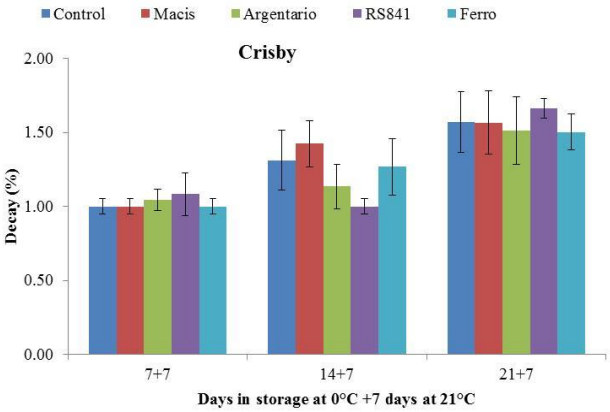

Figure 5. The effects of rootstocks on fungal decay of Crisby watermelon fruits during shelf life

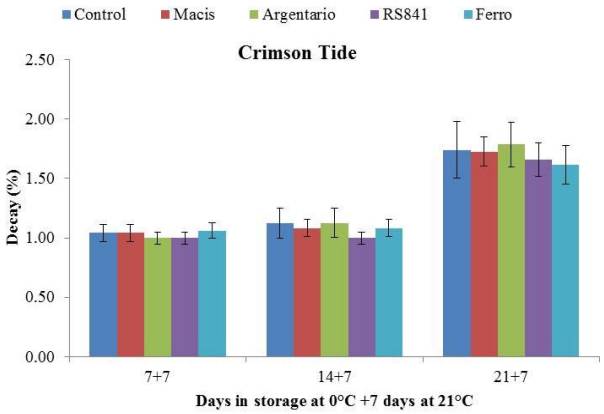

Figure 6. The effects of rootstocks on fungal decay of Crimson Tide watermelon fruits during shelf life

increased susceptibility of fruit to decay due to CI. Risse et al. (1990) reported that most of the decay was observed on the sites of CI at 1 and 7°C and in small watermelon cultivars, most decay was observed from the stem end at 13°C and 21°C. Similar findings were reported in watermelon fruit by Özdemir et al. (2016, 2018).

Flesh firmness decreased during storage and shelf life for both cultivars. Fruit flesh firmness of watermelons grafted on Ferro and RS841 rootstocks were higher than others in CT and CR cultivars during the storage and shelf life (Table 1 and 2). Consistent with our results, Özdemir et al. (2016, 2018) reported the grafted fruit had firmer comparing to control fruit in watermelons during storage or shelf life.

Suárez-Hernández et al. (2016) reported that the some rootstocks retained firmness better than control fruit during storage. It was reported that at harvest, the fruit flesh firmness of grafted watermelon was higher than control fruits (Soteriou and Kyriacou, 2015; Karaağaç et al., 2018). Watermelon fruit flesh firmness did not change or reduced during storage during 4 weeks of storage at 5, 10, 15 or 20°C depending on storage temperature and cultivars (Risse et al., 1990). Depending on cultivar, seasonal variation and harvest maturity, postharvest decline in flesh firmness may compromise fruit quality within 14 days from harvest (Kyriacou and Soteriou, 2015). Özdemir et al. (2016) reported that depending on the rootstock and the scion vary the effects of rootstocks on fruit flesh firmness.

TSS content remained above 10% in fruit of both cultivars throughout storage period (Table 1 and 2), rendering fruit acceptable for perceived sweetness as reported by Kyriacou and Soteriou (2015). In CR cultivar, fruit grafted on Ferro and Argentario rootstocks had higher TSS content after storage period for 21 days at 0 °C, compared to other graft combinations and control. Effect of rootstocks on TSS content was not significant during shelf life (Table 1). In case of CT cultivar, fruit grafted on Ferro and RS-841 rootstocks had higher TSS content during storage, compared to other graft combinations and control. Fruit grafted on RS-841 rootstock had higher TSS content during shelf life, compared to other graft combinations and control (Table 2). Although, some previous studies showed that, grafting on the bottle gourd rootstocks of watermelons raised TSS contents compared to the control fruit (Suárez-Hernández et al., 2016) and grafted watermelons had lower TSS content compared to control (Kyriacou and Soteriou, 2015). In other studies, our reports are consistent with the previous studies, indicating effects of rootstocks on TSS content, cultivar depending Özdemir et al. (2016, 2018).

Juice pH value slightly decreased during the storage and shelf life (Table 1 and 2). In CR cultivar, effect of rootstocks on pH value was not significant during storage and control fruit had higher pH compared to grafted fruit during shelf life (Table 1). In CT cultivar, fruit grafted on Ferro and RS-841 rootstocks had lower pH compared to other grafted fruit and control during storage and effect of rootstocks on pH value was not significant during shelf life (Table 2). Our reports are consistent with the previous studies (Özdemir et al., 2016, 2018).

TA content slightly increased in parallel with changes in juice pH during storage and shelf life for both cultivars during the storage and shelf life (Table 1 and 2). In CR and CT cultivar, fruit on Ferro and RS841 rootstocks resulted in higher TA than those on other rootstocks and control fruit after 21 days of storage and shelf life (Table 1 and 2). Higher TA due to grafting was reported in watermelon fruit (Proietti et al., 2008; Çandır et al., 2013, Özdemir et al., 2016, 2018).

It was found a slight increase during storage and shelf life for both cultivars in ripening (1–7) ratings (Table 1 and 2), indicating fruit became overripe toward the end of storage. Similar findings were reported by Risse et al. (1990) for several watermelon cultivars during 4 weeks of storage at 5, 10, 15 or 20°C. In CR cultivar, fruit grafted on RS841 rootstock had lower ripening scores than those from other rootstocks and control fruit after 21 days of storage and effect of rootstocks on ripening ratings were not significant during shelf life (Table 1). In CT cultivars all grafted fruit had lower ripening scores, compared to control fruit after 21 days of

Table 1. The effects of rootstocks on fruit flesh firmness (N), TSS (%), juice pH, TA (%) and ripening (1–7) of Crisby (CR) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
Firmness (N)	CR (Control)	7.84 b	6.81 c	5.67 c	5.49 c	6.45 d	6.16 d	4.89 c	4.14 c	3.87 c	4.77 c
	CR/Macis	7.57 c	7.40 bc	6.72 b	6.18 b	6.97 c	6.31 cd	6.69 b	5.59 b	5.23 b	5.96 c
	CR/Argentario	8.13 a	7.79 ab	6.74 b	6.37 b	7.26 bc	6.86 bc	6.75 b	6.28 a	5.43 b	6.33 b
	CR/RS841	8.26 a	8.40 a	7.57 a	7.12 a	7.84 a	7.65 a	7.50 a	6.86 a	6.62 a	7.16 a
	CR/Ferro	8.24 a	7.91 ab	6.96 b	7.01 a	7.53 b	7.47 ab	6.80 b	6.86 a	6.55 a	6.92 a
TSS (%)	CR(Control)	10.30 a	10.40 b	11.20 a	10.60 b	10.60 c	10.60 bc	10.60 a	11.10 a	10.80 a	10.80 a
	CR/Macis	10.20 a	10.50 b	11.10 a	10.40 b	10.60 c	10.20 c	10.50 a	10.90 a	10.70 a	10.60 a
	CR/Argentario	10.60 a	10.60 b	11.10 a	11.20 a	10.90 ab	10.80 b	10.20 a	11.00 a	10.90 a	10.70 a
	CR/RS841	10.30 a	10.60 b	11.20 a	11.10 a	10.80 bc	10.90 ab	10.50 a	11.00 a	10.90 a	10.80 a
	CR/Ferro	11.00 a	11.20 a	11.20 a	11.30 a	11.20 a	11.40 a	10.40 a	11.40 a	10.80 a	11.00 a
Juice pH	CR(Control)	5.65 a	5.69 bc	5.69 a	5.57 a	5.65 a	5.80 a	5.65 a	5.66 a	5.74 a	5.71 a
	CR/Macis	5.65 a	5.64 c	5.64 a	5.57 a	5.62 a	5.63 b	5.53 b	5.51 b	5.63 b	5.58 bc
	CR/Argentario	5.67 a	5.74 ab	5.61 a	5.67 a	5.67 a	5.64 b	5.59 ab	5.58a b	5.68 ab	5.62 b
	CR/RS841	5.58 a	5.69 bc	5.60 a	5.58 a	5.61 a	5.62 b	5.51 b	5.49 b	5.55 b	5.54 c
	CR/Ferro	5.54 a	5.82 a	5.58 a	5.66 a	5.65 a	5.65 b	5.54 b	5.54 b	5.63 c	5.59 bc
TA (%)	CR(Control)	0.15 a	0.17 a	0.17 bc	0.16 a	0.16 ab	0.16 b	0.18 a	0.16 b	0.16 b	0.16 b
	CR/Macis	0.15 a	0.15 a	0.16 c	0.14 a	0.15 b	0.16 b	0.16 a	0.16 b	0.17 ab	0.16 b
	CR/Argentario	0.14 a	0.16 a	0.16 c	0.15 a	0.15 b	0.16 b	0.16 a	0.16 b	0.16 b	0.16 b
	CR/RS841	0.15 a	0.17 a	0.18 a	0.16 a	0.17 a	0.18 a	0.18 a	0.18 ab	0.19 a	0.18 a
	CR/Ferro	0.16 a	0.16 a	0.18 a	0.16 a	0.17a	0.17 ab	0.18 a	0.19 a	0.18 a	0.18 a
Ripening (1–7)	CR(Control)	3.70 a	3.60 a	3.40 a	3.70 a	3.60 a	4.00 a	4.50 a	3.60 a	3.70 a	4.00 a
	CR/Macis	3.20 b	3.50 a	3.40 a	3.70 a	3.40 ab	3.80 a	3.40 c	3.70 a	4.10 a	3.80 a
	CR/Argentario	3.60 a	3.40 a	3.50 a	3.90 a	3.60 a	3.80 a	3.80 b	3.80 a	3.90 a	3.80 a
	CR/RS841	3.10 b	3.20 a	3.30 a	3.50 a	3.20 b	3.50 a	3.40 c	3.50 a	3.70 a	3.50 a
	CR/Ferro	3.30a b	3.30 a	3.70 a	3.50 a	3.40 a	3.60 a	3.50 c	3.60 a	3.60 a	3.60 a

[x]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05

Table 2. The effects of rootstocks on fruit flesh firmness (N), TSS (%), juice pH, TA (%) and ripening (1–7) of Crimson Tide (CT) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
Firmness (N)	CT(Control)	7.37 c	6.23 b	6.28 c	5.86 b	6.43 c	5.74 c	6.16 c	5.18 c	4.85 c	5.48 e
	CT/Macis	7.75 bc	6.95 ab	6.08 c	5.89 b	6.67 bc	6.06 c	6.06 c	5.64 bc	5.69 b	5.86 d
	CT/Argentario	7.96 b	7.08 a	6.59 bc	5.91 b	6.88 b	7.03 b	6.74 b	5.85 b	5.86 b	6.37 c
	CT/RS841	8.31 ab	7.60 a	7.13 ab	6.92 a	7.49 a	7.16 b	6.78 b	6.59 a	7.24 a	6.94 b
	CT/Ferro	8.55 a	7.68 a	7.53 a	7.06 a	7.70 a	7.99 a	7.52 a	7.04 a	7.28 a	7.46 a
TSS (%)	CT(Control)	11.10 a	11.10 a	11.20 a	11.10 ab	11.10 a	10.90 a	10.70 b	10.60 a	10.90 b	10.80 b
	CT/Macis	10.60 a	10.90 a	10.70 a	10.30 c	10.60 c	10.60 a	10.70 b	10.40 a	10.80 b	10.60 b
	CT/Argentario	10.80 a	11.20 a	10.50 a	10.60 bc	10.80 bc	10.10 b	10.80 ab	10.60 a	11.00 b	10.60 b
	CT/RS841	10.90 a	11.50 a	10.90 a	10.80 ab	11.00 ab	10.90 a	11.30 a	11.30 a	11.60 a	11.30 a
	CT/Ferro	10.60 a	11.20 a	11.10 a	11.20 a	11.00 ab	10.80 a	10.40 b	10.90 a	10.70 b	10.70 b
Juice pH	CT(Control)	5.67 a	5.63 a	5.71 a	5.72 ab	5.68 a	5.69 a	5.56 a	5.53 a	5.74 a	5.63 a
	CT/Macis	5.66 a	5.64 a	5.64 ab	5.78 a	5.68 a	5.60 a	5.53 a	5.52 a	5.73 a	5.59 a
	CT/Argentario	5.69 a	5.62 a	5.57 bc	5.76 a	5.66 a	5.62 a	5.43 bc	5.58 a	5.70 a	5.58 a
	CT/RS841	5.66 a	5.53 ab	5.48 c	5.55 b	5.55 b	5.59 a	5.50 ab	5.46 a	5.62 a	5.54 a
	CT/Ferro	5.56 a	5.47 b	5.50 c	5.57 b	5.53 b	5.61 a	5.42 c	5.50 a	5.63 a	5.54 a
TA (%)	CT(Control)	0.17 ab	0.19 b	0.16 b	0.17 a	0.17 b	0.17 a	0.18 a	0.18 b	0.17 b	0.18 b
	CT/Macis	0.16 b	0.15 c	0.14 c	0.14 b	0.15 c	0.17 a	0.16 a	0.16 c	0.18 ab	0.17 b
	CT/Argentario	0.16 b	0.17 bc	0.15 bc	0.15 ab	0.16 c	0.16 a	0.18 a	0.16 c	0.17 b	0.17 b
	CT/RS841	0.17 ab	0.19 b	0.18 a	0.17 a	0.18 ab	0.17 a	0.18 a	0.20 a	0.21 a	0.19 a
	CT/Ferro	0.18 a	0.21 a	0.18 a	0.17 a	0.19 a	0.18 a	0.18 a	0.19 ab	0.21 a	0.18 a
Ripening (1–7)	CT(Control)	3.30 a	3.80 a	4.40 a	4.10 a	3.90 a	4.60 a	4.20 a	4.70 a	5.70 a	4.80 a
	CT/Macis	3.30 a	3.70 a	4.10 a	4.20 a	3.80 a	4.40 ab	4.10 a	4.00 a	5.00 b	4.40 b
	CT/Argentario	3.20 a	3.30 a	3.70 b	4.10 a	3.50 b	3.30 b	3.60 b	4.20 a	5.10 b	4.00 c
	CT/RS841	3.10 a	3.40 a	3.60 bc	3.90 a	3.50 b	3.70 b	3.70 b	4.00 a	4.80 bc	4.10 bc
	CT/Ferro	3.10 a	3.20 a	3.30 c	3.60 a	3.30 c	3.30 b	3.30 c	3.80 a	4.40 c	3.70 d

[x]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05.

storage and shelf life. Moreover, in CT cultivar, fruit grafted on Ferro rootstock had lowest ripening scores in all grafted and control fruit after 21 days of storage and shelf life (Table 2). Ripening could be retarded by grafting in watermelon fruit at harvest (Özdemir et al., 2016, 2018). Soteriou et al. (2014) found that as grafting retarded the ripening process, optimum harvest maturity in non-grafted plant was reached 35–40 days post-anthesis (dpa) compared with 40-45 dpa in grafted plants. Similarly, Özdemir et al. (2016) reported that fruit grafted on RS841 and Ferro rootstocks for CR cultivar and fruit grafted on RS841, Argentario and Ferro rootstocks for CT cultivar had the lowest ripening ratings after shelf life period following storage.

In CT cultivar, the citric acid amount ranged between 0.06–0.09% during storage and 0.06% to 0.10% for CT cultivar and the malic acid content ranged from 0.19% to 0.25% for CR cultivar and 0.21% to 0.32% for CT cultivar after 21 days of storage and shelf life (Table 3 and 4). In CR cultivar, fruit grafted on Ferro rootstock had higher citric and malic acid content than those from other rootstocks and control fruit after 21 days of storage and fruit grafted on RS841 and Ferro rootstocks had higher citric and malic acid content than those from other rootstocks and control fruit during shelf life (Table 3). In CT cultivar, fruit grafted on RS841 and Ferro rootstocks and control fruit had higher citric acid content than those from other rootstocks after 21 days of storage and fruit grafted on RS841 rootstock had higher citric acid content than those from other rootstocks and control fruit during shelf life (Table 4). Malic acid was the predominant organic acid for both cultivars. In CT cultivar, fruit grafted on RS841 and Ferro rootstocks had higher malic acid content than those from other rootstocks after 21 days of storage and shelf life (Table 4). In similarly to our findings, it was reported malic acid is the predominant organic acid in watermelon fruit by Özdemir et al. (2016, 2018).

Chilling injury (CI) typically occurs after storage at temperatures <7°C in watermelon fruit (Özdemir et al., 2016, 2018). Symptoms of chilling injury include pitting, decline in flesh color, loss of flavour, off-flavours and increased decay when returned to room temperatures (Suslow, 1997). In our study, CI symptoms such as brownish water-soaked areas covered <25% of rind surface of fruit during the storage and shelf life for all rootstocks for both cultivars. In CR cultivar, external (rind) CI was first observed on fruit grafted on RS841 and Macis rootstocks after 14 days of storage and rind CI was observed on all grafted and control fruit after 21 days of storage. Rind CI was first observed on fruit grafted on Argentario, RS841 and Ferro rootstocks after 7+7 days of shelf life and rind CI was observed on all grafted and control fruit after 14+7 days of shelf life. However, the effect of rootstocks and control fruit on the incidence of rind CI was not significant after 21 days of storage and shelf life in CR cultivar (Table 3). In CT cultivar, rind CI was first observed on all grafted and control fruit (except fruit grafted on Argentario rootstock) after 14 days of storage and rind CI was observed on all grafted and control fruit after 21 days of storage. Rind CI was first observed on fruit grafted on Macis, RS841 and Ferro rootstocks after 7+7 days of shelf life and rind CI was observed on all grafted and control fruit after 14+7 days of shelf life (Figure 7). However, effect of rootstocks and control fruit on the incidence of rind CI was not significant after 21 days of storage and shelf life in CT cultivar (Table 4).

Internal (flesh) CI was first and only observed on control fruit after 21 days of storage in CR cultivar. Flesh CI was first observed on fruit grafted on Argentario rootstock and control fruit after 14+7 days of shelf life in CR cultivar (Figure 8). Fruit grafted on Ferro rootstocks did not exhibit flesh CI symptoms during storage and shelf life in CR cultivar. However, effect of rootstocks and control fruit on the incidence of flesh CI was not significant after 21 days of storage and shelf life in CR cultivar (Table 3).

All grafted and control fruit did not exhibit flesh CI symptoms during storage in CT cultivar. Flesh CI was first observed on fruit grafted on Argentario rootstock and control fruit after 21+7 days of shelf life in CT cultivar. Fruit grafted on Macis, RS841 and Ferro rootstocks were not observed flesh CI symptoms during shelf life in CT cultivar. However, effect of rootstocks and control fruit on the incidence of flesh CI was not significant after 21 days of storage and shelf life in CT cultivar (Table 4). In contrast to our findings, it was reported non-grafted CT and CR or CT and CR grafted onto different rootstocks did not exhibit CI symptoms by Özdemir et al. (2016, 2018). Picha (1986) evaluated three watermelon cultivars for CI at different storage temperatures and durations, and reported less external CI developed in fruit stored at 7°C than at 0°C depending on cultivar. In this study, fruit were stored for 12 days at 7°C without loss of marketable fruit. Our data showed that susceptibility to CI also was dependent on the rootstock used. Our results for 0°C storage and shelf life period were similar to those of Risse et al. (1990).

The effects of grafting on hallow heart were not significant during the storage and shelf life for both cultivars (Table 3 and 4). In similarly to our findings, it was reported that effect of rootstocks on hallow heart was not significant during shelf life by Özdemir et al. (2016). Cushman and Huan (2008) reported that a greater hollow heart ratio in non-grafted watermelon than in grafted watermelon. Moreover, it was reported the environmental and cultural conditions affect incidence of hollow heart beside to rootstocks by Özdemir et al. (2018).

The most abundant sugar was sucrose at the end of the storage time and shelf life in both cultivars (Table 5 and 6). Similar results were reported (Chisholm and Picha, 1986; Kyriacou and Soteriou, 2015; Özdemir et al., 2016, 2018).

Table 3. The effects of rootstocks on citric acid (%), malic acid (%), external (rind) and internal (flesh) chilling injury (CI, 1–5) and hallow heart (1–5) of Crisby (CR) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
Citric acid (%)	CR(Control)	0.08 a	0.07 a	0.08 a	0.10 b	0.08 b	0.07 a	0.07 a	0.07 b	0.08 b	0.08 b
	CR/Macis	0.06 b	0.06 a	0.08 a	0.07 c	0.07 c	0.07 a	0.06 a	0.07 b	0.08 b	0.07 b
	CR/Argentario	0.08 a	0.07 a	0.08 a	0.10 b	0.08 b	0.08 a	0.07 a	0.07 b	0.07 b	0.07 b
	CR/RS841	0.08 a	0.08 a	0.08 a	0.09 bc	0.08 b	0.08 a	0.07 a	0.10 a	0.11 a	0.09 a
	CR/Ferro	0.09 a	0.08 a	0.09 a	0.13 a	0.10 a	0.09 a	0.09 a	0.08 ab	0.11 a	0.09 a
Malic acid (%)	CR(Control)	0.23 b	0.22 a	0.25 b	0.25 bc	0.24 b	0.25 a	0.20 b	0.23 bc	0.25 bc	0.23 b
	CR/Macis	0.24 ab	0.22 a	0.25 b	0.24 c	0.24 b	0.22 a	0.22 b	0.23 bc	0.21 c	0.22 b
	CR/Argentario	0.19 c	0.19 a	0.20 c	0.24 c	0.21 c	0.21 a	0.22 b	0.21 c	0.23 cd	0.22 b
	CR/RS841	0.25 a	0.22 a	0.30 a	0.28 b	0.26 ab	0.25 a	0.29 a	0.26 a	0.28 ab	0.27 a
	CR/Ferro	0.24a b	0.22 a	0.25 b	0.34 a	0.26 a	0.24 a	0.27 a	0.24 ab	0.29 a	0.26 a
CI external (1–5)	CR(Control)	1.00 a	1.00 a	1.00 b	1.46 a	1.11 a	1.00 a	1.00 a	1.29 a	1.23 a	1.13 a
	CR/Macis	1.00 a	1.00 a	1.03 b	1.61 a	1.16 a	1.00 a	1.00 a	1.29 a	1.30 a	1.15 a
	CR/Argentario	1.00 a	1.00 a	1.00 b	1.54 a	1.13 a	1.00 a	1.04 a	1.03 b	1.29 a	1.09 a
	CR/RS841	1.00 a	1.00 a	1.10 a	1.54 a	1.16 a	1.00 a	1.04 a	1.20 ab	1.66 a	1.23 a
	CR/Ferro	1.00 a	1.00 a	1.00 b	1.57 a	1.14 a	1.00 a	1.10 a	1.13 ab	1.21 a	1.11 a
CI internal (1–5)	CR(Control)	1.00 a	1.00 a	1.00 a	1.33 a	1.08 a	1.00 a	1.00 a	1.07 a	1.00 a	1.02 a
	CR/Macis	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.17 a	1.04 a
	CR/Argentario	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.10 a	1.17 a	1.07 a
	CR/RS841	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.33 a	1.08 a
	CR/Ferro	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
Hallow heart (1–5)	CR(Control)	1.23 a	1.22 a	1.43 a	1.37 a	1.31 a	1.23 a	1.53 a	1.50 a	1.43 a	1.43 a
	CR/Macis	1.20 a	1.20 a	1.23 a	1.40 a	1.26 a	1.30 a	1.23 a	1.40 a	1.37 a	1.33 a
	CR/Argentario	1.18 a	1.33 a	1.37 a	1.55 a	1.36 a	1.35 a	1.37 a	1.27 a	1.33 a	1.33 a
	CR/RS841	1.23 a	1.20 a	1.20 a	1.25 a	1.22 a	1.23 a	1.27 a	1.47 a	1.40 a	1.34 a
	CR/Ferro	1.28 a	1.33 a	1.30 a	1.43 a	1.34 a	1.37 a	1.42 a	1.30 a	1.33 a	1.35 a

[x]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05.

Table 4. The effects of rootstocks on citric acid (%), malic acid (%), external (rind) and internal (flesh) chilling injury (CI, 1–5) and hallow heart (1–5) of Crimson Tide (CT) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21 °C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
Citric acid (%)	CT(Control)	0.10 a	0.09 a	0.12 ab	0.10 a	0.10 a	0.10 a	0.07 b	0.08 a	0.07 a	0.08 b
	CT/Macis	0.06 b	0.05 b	0.09 b	0.07 b	0.07 b	0.09 a	0.09 b	0.05 a	0.08 a	0.08 b
	CT/Argentario	0.08 ab	0.08 ab	0.09 b	0.07 b	0.08 b	0.09 a	0.08 b	0.07 a	0.07 a	0.08 b
	CT/RS841	0.10 a	0.09 a	0.14 a	0.09 ab	0.10 a	0.10 a	0.14 a	0.07 a	0.09 a	0.10 a
	CT/Ferro	0.10 a	0.09 a	0.13 ab	0.09 a	0.10 a	0.09 a	0.12 a	0.09 a	0.10 a	0.10 b
Malic acid (%)	CT(Control)	0.23 c	0.23 cd	0.25 b	0.26 ab	0.24 c	0.24 a	0.31 a	0.24 c	0.25 b	0.26 bc
	CT/Macis	0.21 c	0.21 d	0.26 b	0.20 b	0.22 c	0.23 a	0.29 a	0.21 c	0.24 b	0.2 4c
	CT/Argentario	0.27 b	0.26 bc	0.32 a	0.31 a	0.29 b	0.25 a	0.29 a	0.26 bc	0.24 b	0.26 bc
	CT/RS841	0.30 a	0.29 ab	0.36 a	0.32 a	0.32 a	0.24 a	0.32 a	0.29 ab	0.32 a	0.29 a
	CT/Ferro	0.32 a	0.31 a	0.33 a	0.32 a	0.32 a	0.25 a	0.27 a	0.32 a	0.28 ab	0.28 ab
CI external (1–5)	CT(Control)	1.00 a	1.00 a	1.13 a	1.20 a	1.08 a	1.00 a	1.00 a	1.29 a	2.04 a	1.33 a
	CT/Macis	1.00 a	1.00 a	1.13 a	1.47 a	1.15 a	1.00 a	1.13 a	1.29 a	2.13 a	1.39 a
	CT/Argentario	1.00 a	1.00 a	1.00 a	1.17 a	1.04 a	1.00 a	1.00 a	1.17 a	2.23 a	1.35 a
	CT/RS841	1.00 a	1.00 a	1.11 a	1.25 a	1.09 a	1.00 a	1.13 a	1.13 a	2.61 a	1.47 a
	CT/Ferro	1.00 a	1.00 a	1.04 a	1.29 a	1.08 a	1.00 a	1.13 a	1.13 a	2.33 a	1.40 a
CI internal (1–5)	CT(Control)	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	2.09 a	1.27 a
	CT/Macis	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	CT/Argentario	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.13 a	1.03 a
	CT/RS841	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
	CT/Ferro	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
Hallow heart (1–5)	CT(Control)	1.13 a	1.42 a	1.38 a	1.27 a	1.30 a	1.63 a	1.21 a	1.38 a	1.31 a	1.38 a
	CT/Macis	1.03 a	1.50 a	1.50 a	1.25 a	1.32 a	1.21 a	1.21 a	1.13 a	1.42 a	1.24 a
	CT/Argentario	1.10 a	1.21 a	1.21 a	1.08 a	1.15 a	1.09 a	1.04 a	1.29 a	1.49 a	1.23 a
	CT/RS841	1.37 a	1.17 a	1.23 a	1.17 a	1.24 a	1.14 a	1.29 a	1.17 a	1.38 a	1.25 a
	CT/Ferro	1.30 a	1.71 a	1.08 a	1.34 a	1.36 a	1.09 a	1.21 a	1.13 a	1.07 a	1.12 a

[x]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05.

Figure 7. Rind CI on fruit grafted on RS841 (above), Ferro (in the middle) and Argentario (below) rootstocks after 21+7 days of shelf life in CT cultivar

Figure 8. Flesh CI on fruit grafted on Macis (left) rootstock and control fruits (right) after 21+7 days of shelf life in CR cultivar

Table 5. The effects of rootstocks on fructose (%), glucose (%), sucrose (%) total sugar (%) and sensory quality (1–9) of Crisby (CR) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
Fructose (%)	CR(Control)	4.21 a	3.94 a	3.15 a	3.32 bc	3.65 a	3.21 a	3.07 a	3.09 a	3.42 a	3.20 a
	CR/Macis	4.27 a	4.03 a	3.36 a	3.16 c	3.70 a	3.22 a	3.50 a	3.12 a	2.84 a	3.17 a
	CR/Argentario	4.11 a	3.75 a	3.27 a	3.25 bc	3.60 a	2.94 ab	3.28 a	3.26 a	3.32 a	3.20 a
	CR/RS841	3.67 a	3.61 a	3.56 a	3.68 ab	3.63 a	3.15 a	3.37 a	3.43 a	3.45 a	3.35 a
	CR/Ferro	4.05 a	3.88 a	3.58 a	3.96 a	3.87 a	2.73 b	3.60 a	3.31 a	3.66 a	3.32 a
Glucose (%)	CR(Control)	2.59 a	2.32 a	1.90 a	2.13 a	2.23 a	1.66 a	1.60 a	1.72 a	1.87 ab	1.71 a
	CR/Macis	2.74 a	2.52 a	1.94 a	1.94 a	2.29 a	1.66 a	1.54 a	1.78 a	1.61 b	1.65 a
	CR/Argentario	2.62 a	2.30 a	2.05 a	2.13 a	2.28 a	1.55 a	1.86 a	1.80 a	1.90 ab	1.78 a
	CR/RS841	2.27 a	2.23 a	2.21 a	2.33 a	2.26 a	1.54 a	1.61 a	1.96 a	1.92 a	1.76 a
	CR/Ferro	2.43 a	2.28 a	2.31 a	2.48 a	2.37 a	1.32 a	1.90 a	1.85 a	2.12 a	1.80 a
Sucrose (%)	CR(Control)	3.97 a	3.58 a	3.84 a	3.49 a	3.72 a	4.47 a	4.07 a	4.34 a	4.60 a	4.37 a
	CR/Macis	3.76 a	3.40 a	4.00 a	3.54 a	3.67 a	4.85 a	3.95 a	4.18 a	4.14 a	4.28 a
	CR/Argentario	3.84 a	3.63 a	3.80 a	3.85 a	3.78 a	5.27 a	4.26 a	4.01 a	4.56 a	4.53 a
	CR/RS841	4.17 a	3.98 a	3.29 a	3.16 a	3.65 a	4.72 a	4.01 a	3.81 a	4.23 a	4.19 a
	CR/Ferro	3.77 a	3.63 a	3.50 a	4.44 a	3.83 a	4.94 a	3.98 a	4.25 a	4.46 a	4.41 a
Total sugar (%)	CR(Control)	10.76 a	9.83 a	8.88 a	8.94 b	9.61 a	9.34 a	8.73 a	9.14 a	9.88 a	9.28 a
	CR/Macis	10.76 a	9.94 a	9.29 a	8.63 b	9.66 a	9.73 a	8.99 a	9.07 a	8.59 a	9.09 a
	CR/Argentario	10.56 a	9.68 a	9.12 a	9.23 b	9.65 a	9.76 a	9.40 a	9.07 a	9.77 a	9.50 a
	CR/RS841	10.10 a	9.82 a	9.05 a	9.17 b	9.53 a	9.40 a	8.99 a	9.20 a	9.59 a	9.29 a
	CR/Ferro	10.24 a	9.78 a	9.39 a	10.88 a	10.07 a	8.97 a	9.48 a	9.41 a	10.23 a	9.52 a
Sensory quality (1–9)	CR(Control)	8.30 a	8.30 a	7.00 b	5.40 b	7.30 b	8.10 a	7.10 c	6.50 c	5.50 c	6.80 c
	CR/Macis	8.40 a	8.40 a	7.80 a	6.60 b	7.80 a	8.00 a	7.60 ab	6.80 bc	5.80 b	7.10 b
	CR/Argentario	8.00 a	8.30 a	7.20 b	6.90 a	7.60 ab	8.10 a	8.00 ab	6.90 bc	6.10 b	7.30a b
	CR/RS841	8.20 a	8.60 a	8.00 a	7.00 a	7.90 a	8.20 a	7.50 bc	7.00 ab	6.80 a	7.40 a
	CR/Ferro	8.20 a	8.70 a	7.80 a	7.20 a	8.00 a	8.40 a	8.10 a	7.30 a	6.30a b	7.50 a

[X]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05.

Table 6. The effects of rootstocks on fructose (%), glucose (%), sucrose (%) total sugar (%) and sensory quality (1–9) of Crimson Tide (CT) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
Fructose (%)	CT(Control)	2.89 c	2.82 b	2.54 c	3.33 ab	2.90 c	2.81 bc	3.07 a	3.10 ab	3.59 a	3.14 a
	CT/Macis	2.92 bc	2.91 b	3.09 b	2.51 b	2.86 c	2.63 c	3.14 a	2.73 b	3.12 a	2.90 a
	CT/Argentario	3.20 b	3.13 ab	3.48 ab	3.26 ab	3.27 b	3.14 ab	3.42 a	2.83 b	2.96 a	3.09 a
	CT/RS841	3.63 a	3.46 a	3.59 a	3.58 a	3.56 a	3.01 ab	3.54 a	2.86 b	3.44 a	3.21 a
	CT/Ferro	3.58 a	3.46 a	3.35 ab	3.71 a	3.53 ab	3.16 a	3.12 a	3.45 a	3.16 a	3.22 a
Glucose (%)	CT(Control)	1.88 c	1.80 b	1.71 c	2.15 ab	1.88 b	1.79 a	1.81 a	1.70 a	2.13 a	1.86 a
	CT/Macis	2.09 b	1.99 b	1.99 bc	1.57 c	1.91 b	1.75 a	1.88 a	1.55 a	1.83 a	1.75 a
	CT/Argentario	2.05 b	1.97 b	2.06 ab	1.87 bc	1.99 b	1.73 a	1.89 a	1.49 a	1.62 a	1.68 a
	CT/RS841	2.54 a	2.41 a	2.36 a	2.22 ab	2.38 a	1.86 a	2.20 a	1.57 a	2.01 a	1.91 a
	CT/Ferro	2.42 a	2.34 a	2.19 ab	2.38 a	2.33 a	1.88 a	1.80 a	1.75 a	1.80 a	1.81 a
Sucrose (%)	CT(Control)	5.24 a	4.72 a	5.15 a	4.32 a	4.86 a	4.90 a	5.60 ab	5.31 a	4.41 a	5.06 a
	CT/Macis	4.70 b	4.35 a	4.36 a	4.85 a	4.57 ab	4.47 a	6.39 a	4.61 a	5.16 a	5.16 a
	CT/Argentario	4.92 b	4.66 a	4.22 a	4.54 a	4.58 ab	4.35 a	4.68 bc	5.27 a	4.94 a	4.81 a
	CT/RS841	4.39 c	3.91 a	4.31 a	3.88 a	4.12 c	4.86 a	5.01 bc	4.59 a	5.07 a	4.88 a
	CT/Ferro	4.64b c	3.90 a	4.82 a	4.35 a	4.34 bc	4.70 a	3.98 c	5.55 a	4.31 a	4.64 a
Total sugar (%)	CT(Control)	9.81 cd	9.34 a	9.40 a	9.79 a	9.58 cd	9.50 a	10.48 a	10.10 a	10.12 a	10.05 a
	CT/Macis	9.70 d	9.25 a	9.44 a	8.92 a	9.33 d	8.84 a	11.40 a	8.89 a	10.10 a	9.81 a
	CT/Argentario	10.16 c	9.75 a	9.76 a	9.67 a	9.83 bc	9.22 a	9.98 a	9.60 a	9.52 a	9.58 a
	CT/RS841	10.55 a	9.77 a	10.25 a	9.68 a	10.06 ab	9.73 a	10.74 a	9.02 a	10.52 a	10.00 a
	CT/Ferro	10.48 ab	9.70 a	10.35 a	10.44 a	10.24 a	9.73 a	8.90 a	10.73 a	9.27 a	9.66 a
Sensory quality (1–9)	CT(Control)	8.20 ab	8.00 a	7.60 a	7.10 b	7.70 bc	7.60 b	6.60 b	6.40 c	3.90 c	6.10 c
	CT/Macis	7.90 b	8.00 a	7.30 b	7.20 b	7.60 c	7.90 b	7.20 ab	6.90 bc	5.00 b	6.80 b
	CT/Argentario	8.50 a	8.60 a	7.80 a	7.00 b	8.00 ab	8.30 a	7.80 a	7.30 ab	5.90 a	7.30 a
	CT/RS841	8.40 a	8.30 a	7.90 a	8.10 a	8.20 a	8.20 a	7.30 ab	7.00 ab	6.60 a	7.30 a
	CT/Ferro	8.40 a	8.30 a	7.90 a	8.20 a	8.20 a	8.30 a	7.60 a	7.50 a	6.20 a	7.40 a

[X]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05.

Changes in on fructose, glucose, sucrose, and total sugar contents were not significant during storage and shelf life in CR cultivar (Table 5).

In CT cultivar, fructose, glucose and total sugar contents were higher in fruit grafted on RS841 and Ferro rootstocks than those on other grafted and control fruit after 21 days of storage at 0°C and sucrose content was lower in fruit grafted on RS841 and Ferro rootstocks than other grafted and control fruit after 21 days of storage at 0°C (Table 6). Changes in on fructose, glucose, sucrose, and total sugar contents were not significant during shelf life in CT cultivar (Table 6). In previous studies, it was reported an accumulation of sucrose accompanied the decline in total soluble carbohydrates and soluble solids content in grafted and non-grafted watermelons during storage for 14 days at 25°C (Kyriacou and Soteriou, 2015) and Radulovic et al. (2007) reported that a significant decrease total sugar contents of watermelons during storage for 14 days at 20°C. In contrast to our findings, Chisholm and Picha (1986) reported that sucrose, glucose, and fructose concentrations of watermelons mostly did not change during storage for 14 days at 0°C plus 5 days at 23°C, but all generally were reduced at higher storage temperatures. Preservation of sugars at lower storage temperature may be attributed to a presumably lower rate of respiration (Özdemir et al., 2016). In similarly to our findings, in previous studies, between the hybrid rootstocks, mean sucrose concentration was undifferentiated (Kyriacou and Soteriou, 2015). Changes in on total and individual sugar contents were not significant during storage and shelf life (Özdemir et al., 2016). In CR cultivar, effect of grafting on total and individual sugar contents was not significant during storage (Özdemir et al., 2018). In one study, all of the sugars amounts in Crimson Tide watermelon fruit of grafting on the bottle gourd rootstocks enhancement compared to the control fruits and other rootstocks (Çandir et al., 2013). Lower sugar content was reported in grafted watermelon fruit than nongrafted fruit in some studies (Yetişir et al., 2003; Davis and Perkins-Veazie, 2005).

Taste scores (1–9) declined to the lowest level for 21 days of storage at 0°C in CR cultivar (Table 5) and the lowest level during shelf life in CT cultivar (Table 6). Lower taste score may be related to becoming of overripe of control fruit and grafted fruit on Macis and Argentario rootstocks and control fruit. Furthermore, panellists did not detect off-flavors in fruit from grafted plants. As the storage time extended, taste tented to decrease, taste scores of 7.9–8.5 were given to the fruit, which was initially tested by the tasting panellists and decreased to mean scores of >6.1–7.5 during storage at 0°C for 21 days and additional 7 days shelf life at 21°C. But, In CT cultivar, taste scores of control fruit with 3.90 scores decreased to the lowest level at the third week of shelf life. This taste scores in control fruit were found below the acceptability (>5.00) limit (Table 6). In CR cultivar, all grafted fruit higher taste scores than control fruit after 21 days of storage (Table 5). In CT cultivar, fruit grafted on Ferro, RS841 and Argentario rootstocks received higher taste scores than those on Macis rootstock and control fruit after 21 days of storage (Table 6). Bruton et al. (2009) and Özdemir et al. (2016, 2018) reported similar findings with the fruit from grafted watermelons.

Effects of grafting on flesh color lightness (L* value) was not significant at the end of the storage time and additional 7 days shelf life at 21°C for both cultivars (Table 7 and 8). In contrast to our findings, in previous studies, flesh color lightness decreased during storage and/or shelf life in CR and CT cultivar Özdemir et al. (2016, 2018). Perkins-Veazie and Collins (2006) determined lower flesh color L* values in the fruit after 14 days of storage at 21°C, compared freshly harvested watermelons. Kyriacou and Soteriou (2015) reported that flesh color lightness of watermelon fruit was affected by rootstock and storage and all hybrid rootstocks invariably maintained darker flesh color during storage.

In CR cultivar, flesh color C* value peaked after 7 days and then decreased during storage (Table 7). In CT cultivar, flesh color C* value showed gradual decrease toward the end of storage (Table 8). In CR fruit, fruit grafted on RS841 and Ferro rootstocks had more compact (higher C*) color than those on other rootstocks and control fruit during storage and shelf life (Table 7). In CT fruit, during the storage, fruit grafted on Ferro, RS841 and Argentario rootstocks had higher flesh color C* value than control fruit and grafted on Macis rootstock.

The effect of rootstocks on flesh color C* value was not significant during shelf life fruit grafted on Ferro, RS841 and Argentario rootstocks had higher flesh color C* value than those on Macis and control fruit during storage and effect of rootstocks on flesh color C* value was not significant during shelf life (Table 8). In similarly to our findings in CR cultivar, Özdemir et al. (2016) reported that flesh color C* value continuously decreased during shelf life period at 21°C following storage at 7°C in CR and CT (except our findings in CT cultivar). Özdemir et al. (2018) reported similar findings with the fruit from grafted watermelons.

The flesh color h° values showed a progressive increase in non-grafted fruit with a lesser extent in grafted fruit during storage in both cultivars (Table 7 and 8). This indicated a change of flesh color from red to orange-yellow. These changes in h° value indicate over-ripening and senescence of watermelons which are subjected to prolonged storage (Kyriacou and Soteriou, 2015). In CR cultivar, effect of rootstocks on flesh color h° value was not significant after 21 days of storage, but control fruit had higher flesh color h° values than grafted fruit during shelf life (Table 7). In CT cultivar, fruit grafted on Macis and Argentario rootstocks and control fruit had higher flesh color h° values than

Table 7. The effects of rootstocks on fruit flesh color (L*, C* and h°), total lycopene (µg g^{-1}) and beta carotene (µg g^{-1}) of Crisby (CR) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
L*	CR(Control)	38.16 a	42.98 a	44.94 a	43.87 a	42.49 a	42.83 a	41.91 a	43.52 a	45.39 a	43.41 a
	CR/Macis	36.84 a	42.28 a	44.01 a	44.77 a	41.97 a	41.56 a	40.57 a	42.64 a	42.04 a	41.70 a
	CR/Argentario	37.07 a	44.02 a	43.16 a	43.12 a	41.84 a	44.02 a	40.03 a	43.24 a	42.76 a	42.52 a
	CR/RS841	39.13 a	42.32a a	41.45 a	41.84 a	41.19 a	41.10 a	41.86 a	42.14 a	42.14 a	41.81 a
	CR/Ferro	38.37 a	41.88 a	42.37 a	44.99 a	41.90 a	41.48 a	40.16 a	42.60 a	43.67 a	41.98 a
C*	CR(Control)	28.32 a	31.31 b	28.72 d	28.71 b	29.26 b	32.75 b	34.51 a	29.40 cd	29.15 b	31.45 c
	CR/Macis	28.88 a	31.49 b	32.94 c	29.36 b	30.86 b	33.32 b	36.42 a	29.11 d	31.11 a	32.49 bc
	CR/Argentario	28.19 a	31.71 b	32.52 b	30.41 b	30.95 b	32.58 b	35.22 a	30.60 bc	32.18 a	32.75 bc
	CR/RS841	29.10 a	37.09 a	35.77 a	34.58 a	34.14 a	35.76 a	35.67 a	33.61 a	32.27 a	34.33 a
	CR/Ferro	30.32 a	35.81 a	34.72 b	30.86 b	32.93 a	35.86 a	34.56 a	32.66 ab	32.08 a	33.79a b
h°	CR(Control)	38.17 a	45.76 a	46.28 ab	48.22 a	44.61 a	44.08 a	45.87 a	47.49 a	47.44 a	44.69 a
	CR/Macis	35.86 b	45.59 a	47.35 a	46.55 a	43.84 a	44.27 a	44.59 a	46.10 a	45.15 bc	43.76 b
	CR/Argentario	36.76 b	44.37 a	46.91 a	47.37 a	43.85 a	44.58 a	43.43 a	45.28 a	45.85 ab	43.87 ab
	CR/RS841	35.47 b	45.05 a	45.60 b	47.13 a	43.31 a	42.72 b	43.80 a	45.33 a	43.58 c	42.27 c
	CR/Ferro	35.92 b	44.05 a	45.26 b	47.22 a	43.12 a	42.41 b	42.87 a	44.84 a	45.12 bc	43.17 bc
Lycopene (µg g^{-1})	CR(Control)	40.38 a	37.26 a	28.32 b	23.01 a	32.24 a	27.84 c	38.88 a	23.55 c	20.49 a	27.69 b
	CR/Macis	46.25 a	43.90 a	27.42 b	22.50 a	35.02 a	30.23 bc	38.13 a	24.65 c	20.98 a	28.59 b
	CR/Argentario	40.52 a	35.16 a	26.84 b	24.59 a	31.78 a	31.31 bc	34.91 a	26.48 bc	17.85 a	17.64 b
	CR/RS841	43.02 a	38.27 a	32.67 a	28.21 a	35.54 a	38.88 a	44.65 a	33.62 a	20.14 a	34.32 a
	CR/Ferro	42.10 a	36.63 a	32.38 a	28.64 a	34.94 a	34.38 ab	41.81 a	30.49 ab	18.95 a	31.41 ab
Beta carotene (µg g^{-1})	CR(Control)	0.17 a	0.14 a	0.18 a	0.12 a	0.15 a	0.21 a	0.18 a	0.18 a	0.18 a	0.19 a
	CR/Macis	0.13 a	0.11 a	0.18 a	0.12 a	0.14 a	0.21 a	0.19 a	0.15 a	0.16 a	0.18 a
	CR/RS841	0.18 a	0.15 a	0.15 a	0.12 a	0.15 a	0.24 a	0.16 a	0.14 a	0.10 a	0.16 a
	CR/Argentario	0.23 a	0.21 a	0.18 a	0.11 a	0.18 a	0.28 a	0.22 a	0.15 a	0.15 a	0.20 a
	CR/Ferro	0.17 a	0.16 a	0.18 a	0.15 a	0.17 a	0.33 a	0.20 a	0.20 a	0.18 a	0.23 a

[X]Mean separation was performed by Fisher's LSD test. Means (n= 3) followed by same letters within a column are not significantly different at P<0.05.

Table 8. The effects of rootstocks on fruit flesh color (L*, C* and h°), total lycopene (µg g^{-1}) and beta carotene (µg g^{-1}) of Crimson Tide (CT) watermelon fruits during storage at 0°C and following 7 days at 21°C

Parameters	Scion/ rootstock	Days in storage at 0°C				Mean	Days in shelf life at 21°C				Mean
		0	7	14	21		0+7	7+7	14+7	21+7	
L*	CT(Control)	40.46 a	41.85 a	43.09 a	45.35 a	42.69 a	45.55 a	43.57 a	29.06 a	40.76 a	39.73 a
	CT/Macis	43.08 a	44.15 a	42.10 a	44.82 a	43.54 a	44.36 a	42.48 a	27.37 a	41.35 a	38.89 a
	CT/Argentario	40.73 a	43.11 a	42.36 a	46.94 a	43.29 a	40.83 a	41.65 a	27.89 a	43.35 a	38.43 a
	CT/RS841	43.91 a	42.65 a	42.10 a	46.21 a	43.72 a	41.52 a	40.64 a	30.94 a	45.71 a	39.71 a
	CT/Ferro	39.17 a	42.03 a	43.92 a	43.85 a	42.24 a	41.60 a	43.38 a	30.21 a	45.51 a	40.17 a
C*	CT(Control)	33.91 b	32.95 a	31.29 c	30.99 b	32.29 c	35.27 c	32.19 c	16.99 a	25.02 b	27.37 a
	CT/Macis	33.99 b	34.01 a	34.71 b	31.84 b	33.64 b	37.01 b	33.98 bc	18.76 a	26.92 b	29.17 a
	CT/Argentario	37.07 a	33.76 a	34.63 b	34.29 a	34.94 a	39.74 a	38.01 a	18.49 a	30.81 a	31.76 a
	CT/RS841	36.25 a	34.93 a	36.55a b	34.60 a	35.58 a	39.10 a	33.82 bc	17.69 a	31.39 a	30.50 a
	CT/Ferro	37.35 a	34.27 a	37.76 a	35.27 a	36.16 a	36.98 a	35.40 b	17.38 a	33.05 a	30.70 a
h°	CT(Control)	39.61 a	42.50 a	44.37a b	46.49 a	43.24 a	45.22 a	43.65 ab	44.48 a	45.42 a	44.69 a
	CT/Macis	40.59 a	42.40 ab	44.52 a	46.61 a	43.53 a	43.77 ab	44.48 a	42.48 a	44.33 a	43.76 ab
	CT/Argentario	40.29 a	41.78 b	43.08 c	46.45 a	42.90 ab	42.72 b	44.22 ab	43.00 a	45.52 a	43.86 ab
	CT/RS841	40.63 a	41.03 c	43.08 c	44.68 ab	42.35 bc	42.41 b	40.67 c	42.36 a	43.64 a	42.27 c
	CT/Ferro	39.75 a	40.76 c	43.20 bc	43.88 b	41.90 c	41.84 b	41.95b c	44.54 a	44.37 a	43.17 bc
Lycopene (µg g^{-1})	CT(Control)	34.53 c	30.03 c	25.77 c	31.37 b	30.42 b	35.41 c	32.50 a	31.06 b	35.74 b	33.68 c
	CT/Macis	33.08 c	30.00 c	32.18 bc	24.63 c	29.97 b	35.27 c	32.49 a	36.70 b	35.48 b	34.98 c
	CT/Argentario	55.10 a	52.68 a	36.02 ab	32.89 ab	44.17 a	42.99 ab	34.64 a	35.62 b	39.10a b	38.09 b
	CT/RS841	45.05 b	43.25 b	44.02 a	36.92 ab	42.31 a	40.11 b	36.21 a	45.00 a	41.74 a	40.76 ab
	CT/Ferro	46.89 b	44.15 b	41.42 a	38.69 a	42.79 a	46.00 a	39.44 a	45.16 a	43.12 a	43.43 a
Beta carotene (µg g^{-1})	CT(Control)	0.16 a	0.12 a	0.18 a	0.11 a	0.14 a	0.15 a	0.11 a	0.11 a	0.10 a	0.12 a
	CT/Macis	0.17 a	0.12 a	0.13 a	0.14 a	0.14 a	0.17 a	0.09 a	0.10 a	0.16 a	0.13 a
	CT/Argentario	0.19 a	0.17 a	0.14 a	0.11 a	0.15 a	0.12 a	0.17 a	0.12 a	0.09 a	0.13 a
	CT/RS841	0.30 a	0.09 a	0.06 a	0.08 a	0.13 a	0.14 a	0.10 a	0.09 a	0.10 a	0.10 a
	CT/Ferro	0.16 a	0.14 a	0.11 a	0.06 a	0.12 a	0.11 a	0.06 a	0.13 a	0.10 a	0.10 a

[X]Mean separation was performed by Fisher's LSD test. Means (n=3) followed by same letters within a column are not significantly different at P<0.05.

other grafted fruit during storage and shelf life (Table 8). Flesh color changes were observed in the fruits, suggesting that fruit ripening occurs faster in control fruits than grafted fruit during storage. Özdemir et al. (2018) reported similar findings with the fruit from grafted watermelons. Watermelon flesh color varies from brilliant red (poor flesh color h°) to orange red (top flesh color h°) as ripening level progresses. Özdemir et al. (2016) reported that grafted and non-grafted fruit showed a progressive increase in flesh color h° value after shelf life period following storage, indicating a shift from red to orange-yellow. This changes in flesh color h° value, characteristic of over-ripening and senescence has been reported after prolonged postharvest storage of watermelons (Kyriacou and Soteriou, 2015).

Lycopene content in both cultivars showed similar trend with flesh color C* values (Table 7 and 8). Lycopene content significantly decreased at the end of storage for both cultivars. In CR cultivar, effect of rootstocks on lycopene content was not significant after 21 days of storage, but fruit grafted on RS841 and Ferro rootstocks had higher lycopene content than grafted fruit during shelf life (Table 7).

In CT cultivar, fruit grafted on RS841, Argentario and Ferro rootstocks had higher lycopene content than those on Macis rootstock and control fruit after 21 days of storage and fruit grafted on RS841 and Ferro rootstocks had higher lycopene content than those other rootstock and control fruit during shelf life (Table 8). It was reported that grafted plants higher lycopene content than non-grafted watermelon fruit during storage. (Kyriacou and Soteriou, 2015). The overall intensity of flesh color (C* value), hue angle (h° value) and lycopene content were impressed by storage time and rootstocks (Özdemir et al., 2016). The increase in flesh color C* value of watermelon fruit was probably as a result of the increase in lycopene content (Perkins-Veazie and Collins, 2006). Postharvest color changes and lycopene biosynthesis in watermelons can be affected by storage temperature and cultivar (Özdemir et al., 2018). Perkins-Veazie and Collins (2006) reported that watermelons stored at 21°C had higher flesh color C* value and lycopene content, compared to initial value at harvest whereas no or little change was observed in flesh color C* value and lycopene content of fruit held at 5°C or 13°C depending on cultivars. Degradation in lycopene during senescence of non-grafted watermelon fruit and grafted fruit after prolonged storage and consequent shelf life period led to decrease in flesh color C* value and increase in flesh color h° value (Özdemir et al., 2018).

Effects of grafting on β-carotene content were not significant at the end of the storage time and shelf life for both cultivars (Table 7 and 8). Özdemir et al. (2016, 2018) reported similar findings with the fruit from grafted watermelons. Perkins-Veazie and Collins (2006) reported that watermelons stored for 14 days at 21°C gained 50-139% in β-carotene compared to fresh fruit, whereas fruit held at 5 and 13°C changed little in β-carotene content. In our study, β-carotene content decrease during storage and shelf life.

4. Conclusions

The CI areas covered <25% of rind surface of fruit for both cultivars. The effect of rootstocks and control fruit on the incidence of rind and flesh CI was not significant after 21 days of storage and shelf life in CR and CT cultivars. Weight loss in grafted and control fruit were very low (<1%) during storage for both cultivars. Fungal decay was not observed during storage for both cultivars. However, it was observed during the shelf life.

The decayed areas covered <10% of rind surface of fruit. The graft combinations did not differ in the incidence of fungal during shelf life for both cultivars. TSS content remained above 10% in fruit of both cultivars throughout storage period. TA content slightly increased in parallel with changes in juice pH during storage and shelf life for both cultivars at the end of the storage time and additional 7 days shelf life at 21°C. The citric acid content from organic acids changed from 0.06% to 0.09% for CR cultivar and 0.06% to 0.10% for CT cultivar and the malic acid content changed from 0.19% to 0.25% for CR cultivar and 0.21% to 0.32% for CT cultivar after 21 days of storage and shelf life. The effects of grafting on hallow heart were not significant during the storage and shelf life for both cultivars. The most abundant sugar was sucrose at the end of the storage time and shelf life in both cultivars.

Taste scores (1–9) declined to the lowest level for 21 days of storage at 0°C in CR cultivar and the lowest level during shelf life in CT cultivar. Effects of grafting on flesh color lightness (L* value) was not significant at the end of the storage time and shelf life for both cultivars. The flesh color h° values showed a progressive increase in non-grafted fruit with a lesser extent in grafted fruit during storage in both cultivars. Lycopene content significantly decreased at the end of storage for both cultivars.

Effects of grafting on β-carotene content were not significant during the storage at 0°C for 21 days and additional 7 days shelf life at 21°C for both cultivars. During the storage and shelf life, watermelons grafted on Ferro and RS841 rootstocks retained fruit flesh firmness, compared to the non-grafted fruit for both cultivars.

Watermelons grafted on Ferro and RS841 rootstocks had higher flesh color with lower ripening and softening and higher lycopene content for CR and/or CT fruit during shelf life. Taste scores of grafted fruit had scored higher than control fruits. Watermelons could be kept for 7 days at 0 °C without rind and flesh CI.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Effects of Prohexadione-Calcium on 'Monroe/GF 677' Peach Vegetative Shoot Growth, Fruit Yield and Quality

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Shoot length

Abstract

Prohexadione-calcium (Pro-Ca) is a recently developed plant growth retardant used in some fruit trees. However, it is important that the reduction of vegetative shoot growth does not decrease yield and fruit quality. In this study, the effects of Pro-Ca applications on the vegetative growth and some fruit quality of the Monroe peach, growing on vigor rootstock (GF 677) were investigated. For this reason, trees were sprayed twice with 0 (water + surfactant), 62.5, 125, 250 g 100 L^{-1} water Pro-Ca in the annual shoots reached 5 cm within a three week interval in the spring of 2018 and 2019 years. Shoot length was decreased by 28-32% for shoots treated with Pro-Ca. The average internode length was significantly reduced for Pro-Ca-treated shoots. The lowest average internode length on the shoot was obtained with 125 and 250 g 100 L^{-1}. The effects on fruit quality were positive in this study. Application of Pro-Ca (125 and 250 g 100 L^{-1}) increased the fruit size and fruit mass of cv. Monroe' peach. 250 g 100 L^{-1} Pro-Ca concentration led to firmer fruit relative to the other applications and control fruits. Two application 125 or 250 g 100 L^{-1} Pro-Ca applications were found more effective considering the criteria investigated. The Pro-Ca applications were found to be effective in controlling the vegetative shoot growth and fruit quality in cv. Monroe/GF-677 peach.

1. Introduction

Prohexadione calcium (3, 5-dioxo-4-propionylcyclohexanecarboxylic acid) is a plant growth regulator used by dioxygenase enzymes (GA20-oxidase and GA3-oxidase) for its ability to inhibit gibberellin biosynthesis (GAs) by blocking 3β-Hydroxylation (Beam et al., 2002; Davies, 2004). Prohexadione calcium (Pro-Ca) was first tested in rice (*Oryza sativa*) and it was proven to shorten the length of rice by 66-78% by inhibiting gibberellic acid production (Nakayama et al., 1990). Pro-Ca, a research subject in rice, apples, pomes, petunias, and various vegetables, grain crops, etc. many products (Lee et al., 1998; Costa et al., 2004; Ilias and Rajapakse, 2005; Ergun et al., 2007; Kim et al., 2007; Başak 2020; Treadway, 2020), is used extensively in fruit trees, especially by apple producers, instead of mechanical pruning. As it is known, mechanical pruning is a cultural practice with a lot of labor and cost every year (Byers and Yoder 1999; Costa et al., 2001; Uselis et al., 2020; Treadway, 2020). In recent years, the use of Pro-Ca plant growth regulator has increased gradually in terms of decreasing the labor intensity, as orchards have been started to be established by using high density planting and different training systems in other fruit types besides apples. Kaur et al. (2020) applied Pro-Ca (100, 200, 400 mg L^{-1}) and Paclobutrazol (100, 250, 500 mg L^{-1}) plant growth regulators to the leaves of the pear varieties 'Patharnakh' and 'Punjab Beauty' that they grow

with the Y-Trellis training system and high density planting, thus preventing vegetative growth and the workload by requiring less mechanical pruning. They reported that it decreased considerably, especially in orchards with high density planting and especially the application of 400 mg L^{-1} Pro-Ca gave the best results in both low shoot growth and fruit quality. Although Pro-Ca applications have been tested on both vegetable seedlings and fruit trees, the number of researches for peach is almost non-existent. Byers and Yoder (1999) reported that application of Pro-Ca had not effect on shoot length or fruit quality in 'Redhaven' peach and its data were not recorded. However, in most European countries, peach orchards are established as high density planting, which increases the more labor in both mechanical pruning and thinning. In Turkey, the cultivation of peach is increasing with high density planting and also with new training systems. Peach production in Turkey 6th-7th in the world in previous years. It ranks 5th in the world with 789457 tons according to the latest data (FAO, 2020). Peach produced in nurseries in Turkey are on peach seedlings (85%) or more GF 677 rootstocks (10%). However, the use of tall trees results in lower labor efficiency (harvest, pruning, and thinning), and higher use of agro-chemicals per hectare. In addition, it is necessary to reduce the labor force and cost in pruning in peach orchards that are high density planted with new training systems (Hossain et al., 2006). Plant height reduction is playing an important role promoting both yield and quality, and in decreasing cost, area and labor (Hayashi et al., 2001; Karlovic et al., 2004). For this reason, it is important that reduction of this peach tree's vegetative shoot growth concentrations not decrease the yield and fruit quality. So far in Turkey and in the world, some chemicals used in pruning peach trees gibberellin inhibit the biosynthesis of plant growth regulators (chlormequat chloride, daminozide, mefluidide, paclobutrazol, etc.), were studied. It has been reported that these practices prevent shoot development but have no negative or no effect on fruit quality (Davis et al., 1991; Bilginer et al., 1995). However, Pro-Ca, which is used for chemical pruning of fruit types such as apple and pear, has not been studied in peach. Therefore, the main purpose of this research is to determine the efficiency of Pro-Ca plant growth regulator as chemical pruning instead of mechanical pruning. The other purpose of this research was to determine the optimum concentrations in the application of Pro-Ca for controlling the vegetative growth of peach trees, as well as the effects of Pro-Ca on fruit quality.

2. Material and Methods

The experiments were carried out in the Fruits Research Institute (Eğirdir, Isparta, Turkey, 37°49' N latitude and 30°52' E longitude and 926 m above sea level) between 2018 - 2019. To determine the effect of Pro-Ca on vegetative growth control and fruit quality parameters of peaches (*Prunus persica* L.). Uniform 6-years old 'Monroe' peach trees grafted on GF 677 rootstocks (5 × 3 m) were selected and grouped into four blocks with 32 trees in each, based on proximity in the orchard and crop load. The experimental design was a randomized block, with four treatments and four replicates, using a double tree for each treatment. The trees were trained to an open vase and pruned in early spring, and standard cultural practices had been used on the trees for several years. The trees were sprayed twice with 0 (water + surfactant), 62.5, 125, 250 g 100 L^{-1} water Pro-Ca 10% in the annual shoots 5 cm within a three week interval in the spring (1st and 2nd years). All the spray solutions contained 'Spur' as a surfactant [1%, v v^{-1} (Sumi-Agro, Turkey)]. The pulverized treatments were applied with a low pressure hand sprayer.

2.1. Assessment of vegetative growth

The following measurements of plant growth were made during the two seasons of study after autumn leaf fall.

Tree height (cm): The distance from the grafting point of the trees in each application to the tip of the peak branch was measured with a tape measure.

Tree width (cm): The distance from the shoulder level of the canopy of the trees in each application to the tip of the end shoots on both sides parallel to the ground was measured with a tape measure.

Trunk diameter (mm): The trunk diameter of the trees in each application, which is 10 cm high from the grafting point, was measured with a caliper.

Average number of shoots: One-year shoots of the trees in each application were counted and recorded.

Average length of annual shoots (cm): The length of three shoots taken from four directions of the trees was measured with a ruler.

Average diameter of annual shoots (mm): The diameters of three shoots taken from four directions of the trees were measured with calipers from the middle part.

Average number of nodes and average internode length on shoots (cm): The internodes of three shoots taken from four directions of the trees in each application were counted and the average internode length was found by dividing the total node number by the shoot length.

Shoots crotch angle (°): The angle of each shoot with the main branch of three shoots taken from four directions of the trees in each application was recorded.

Phytotoxicity and side effects: Macroscopic observations were made on the shoot, leaves and fruit a few days after the application and until the end of the experiment in order to determine the phytotoxic effects of Pro-Ca applications on leaves, shoots and fruits. In addition, it was observed

whether the applications had an effect on bee activity.

2.2. Assessment of fruit yield and quality

Yields belonging to all applications were obtained by gathering fruits at the appropriate harvest time and weighing (kg tree^{-1}). Some fruit quality characteristics were determined in 20 fruits taken from the lower and upper four sides of the tree from each repetition of each application. Fruit width and length (mm) were measured with the help of a digital caliper, and fruit weight (g) was measured with a digital scale with 0.01 precision. Fruit flesh firmness (N) with Lyoyd Instruments LF Plus brand texture device using 8 mm diameter tip. Fruit color determined with Model CR-300, Minolta in L *, a *, b *, C * and hue° (h°). The fruit surface color was made as % in the surface color formation on the fruit, and the evaluation was in the range of 1-100%. The red fruit group was 100% and the lightest fruit group was considered as 1%, and was examined by five separate panels. The water soluble solid content (SSC, %) of the fruits was recorded with a digital refractometer (Palette PR-32 Atago)]. Titratable acidity (TA, %) and pH were measured with an automatic titrator (Mettler Toledo T50).

2.3. Statistical analysis

The collected data were subjected to statistical analysis using a randomized complete block design. Statistical analyses were performed using SAS-JMP 8.0 package. Mean values were compared using LSD's multiple range test at p<0.05 level. Variables were presented as Mean and Standard deviation.

3. Result and Discussion

3.1. Vegetative growth

In the vegetative measurements which were held in the first year of Pro-Ca applications, it was determined that the tree height, tree width and the trunk diameter was insignificant statistically, but in the second year, it was determined that only the tree width was significant (p<0.05) (Table 1). The tree width was increased in the second year and the maximum tree width was occurred in the 62.5 g 100 L^{-1} and 250 g 100 L^{-1} Pro-Ca applications. When we consider the second year changes' in the tree widths and specially trunk diameters beside the first year, it was revealed that the trees continued to improve not lengthwise, but the in width. The reason of this result can be conceivable as the angles which the shoots made with the branches are increased. Thus, in the second year of our research, the angle that the shoots made with the branch was increased, which this situation is a needed physiological development in fruit growing as the Beyazıt et al. (2012) also stated. The shoot pruning and bending processes provided the angles to widen creates a positive impact on flower buds, increases the fruit quality and controls tree development. In our research, the angle which shoot makes with the main branch was widened and hence, it was determined that the tree width was increased.

In the ANOVA, which was made on the shoot growth, Pro-Ca concentrations had no effect on the average number of shoots statistically in both years (Figure 1A). However, it had effect on the average length of shoots (Figure 1B; P<0.05). The length of shoots decreased compared to the control group in both years. In the first year, the least shoot length was determined as 20.99 cm with 250 g 100 L^{-1}. But in the second year, it was 16.74 cm with concentration of 62.5 g 100 L^{-1} (Figure 1B). Statistically, the Pro-Ca concentrations on average diameter of shoots were significant in 2018, but were not in 2019. The average diameter of shoots increased with the Pro-Ca concentrations in 2018. The concentration 62.5 g 100 L^{-1} had the most average diameter of shoots (4.29 mm) (Figure 1C). The shoot angles differences in applications was significant in 2018 (P<0.05), but they were insignificant in 2019 (Figure 1D). However, the Pro-Ca in the second year made wider angles than the first year. We can say that the applications which were made consecutively widened the shoots angles. In 2018, it was determined that the angle increases with the Pro-Ca concentrations increase as well, and the widest angle was in the use of 250 g 100 L^{-1} (Figure 1D). Studies regarding the Pro-Ca were generally carried out to bring under control the vegetative development of the species such as apples (Schupp et al., 2003; Karlovic et al., 2004; Greene, 2008; Duyvelshoff and Cline, 2013;

Table 1. Effect of Pro-Ca on tree length, canopy width and trunk diameter in Monroe/GFF 677.

Pro-Ca concentrations	Tree length (cm)		Canopy width (cm)		Trunk diameter (mm)	
(g 100 L^{-1})	1st year	2nd year	1st year	2nd year	1st year	2nd year
Control	321.25±19.3	263.50±0.11	165.00±14.7	214.25±0.20ab*	66.56±12.5	114.72±12.81
62.5	276.25±22.9	285.00±0.17	161.25±27.2	239.00±0.13a	54.63±3.01	102.29±9.19
125	302.50±67.0	264.25±0.10	172.50±51.9	195.50±0.26b	53.37±14.4	91.68±7.48
250	270.00±8.16	255.00±0.24	143.75±24.3	233.00±0.23a	55.08±2.90	105.44±13.04
P values	0.228	0.374	0.651	0.021	0.245	0.128

Data were mean ± standard deviation.
Different letters indicate statistically significant differences among the treatments (LSD, P<0.05).

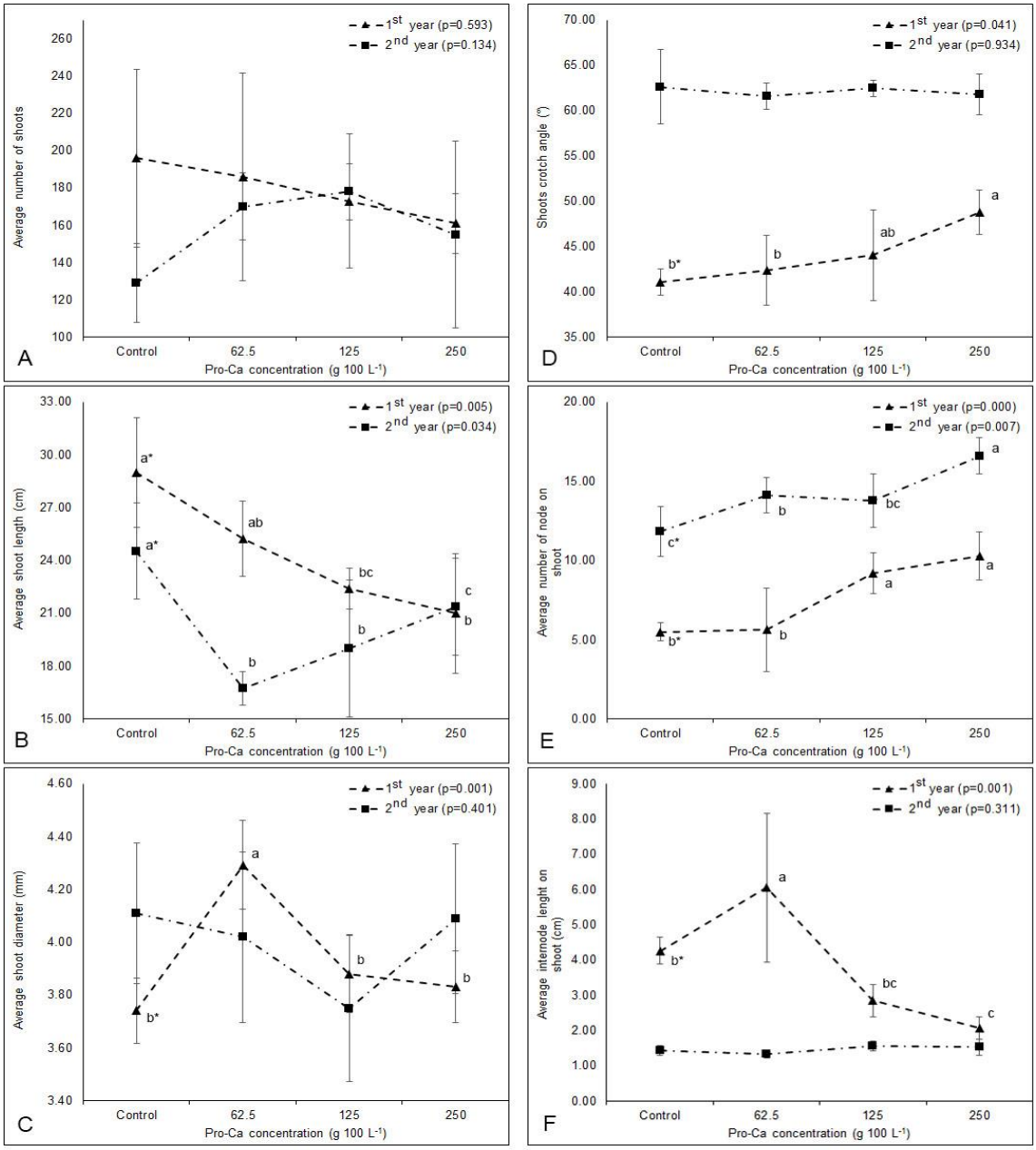

Figure 1. The effect of Pro-Ca implementations on Monroe/GFF 677 peach shoot growth (2018 and 2019).
Data were mean ± standard deviation. Different letters indicate statistically significant differences among the treatments (LSD, P<0.05).

Amarante et al., 2020; Uselis et al., 2020), pears (Elfving et al., 2003; Sugar et al., 2004; Einhorn et al., 2014; Carra et al., 2017; Kaur et al., 2020), and vegetables (Kofidis et al., 2008; Özbay and Ergun, 2015; Başak, 2020), and also to partially downgrade the growth. However, no studies have been made for peach species, and in a study carried out it is observed that no data has been indicated. In current study, Pro-Ca reduced the average shoot length by 30% compared to the control group in the both years. Pro-Ca was effective statistically significant on node number (P<0.05) (2018-2019). In the both years, the average number of nodes increased in accordance with increasing Pro-Ca concentrations (Figure 1E).

The average internode lengths differences were statistically significant in the first year (P<0.05), and insignificant in the second year. Internode lengths was inclined to decrease and increase in the both years. (Figure 1F). In the study, the average number of nodes was increased as the average lengths of shoots decreased. In accordance with our findings, Çetinbaş et al. (2015) noted that the average number of nodes increased and the length of internodes decreased with Pro-Ca in Strakrimson/MM111. This situation can be thought to increase fruit yield, with more flower buds.

3.2. Phytotoxicity and side effects

The macroscopic observations conducted on the shoots, the leaves and fruits a few days after application and until end of trial no phytotoxicity or side effect of Pro-Ca applications could be observed

in both of the years. Besides, it was also determined that the application has no negative effect on bee activity.

3.3. Yield and fruit quality

The effect of Pro-Ca to yield was statistically significant in the both years (P<0.05) (Figure 2A). Yield increased with the applications, and the optimal yield was achieved with the 62.5 g 100 L^{-1} Pro-Ca concentration (23.38–27.04 kg $tree^{-1}$) in the both years. The other high concentration yields, approximately the same values and in the same statistical group were found (Figure 2A). Kaur et al. (2020) reported that 'Punjab Beauty' and 'Patharnakh' pears exhibit a positive reaction to Pro-Ca and increase in yield by 33% to 46% with the 200 and 400 mg L^{-1} concentrations due to the light which penetrates the trees. Hence, our findings demonstrated similar results and increase in the yield by 31% to 50% in the first year, and 31% to 43% percent were recorded.

The results of the ANOVA of one of the most important parameters for assessing fruit quality; fruit width, length, and weight, was statistically significant for the year 2018 (P<0.05), and insignificant for 2019 (Figure 2B, C, D). In the first year, Pro-Ca increased fruit size generally, except the 250 g $100L^{-1}$ reduced fruit size compared to the control group. Nonetheless, it was observed that this has no negative effects on fruit size effectively (Figure 2D).

In many studies in which Pro-Ca applications were carried out, different results indicated on fruit size (Costa et al., 2004; Medjdoub et al., 2004; Kaur et al., 2020). Similarly to our study, Schupp et al. (2003) stated that 250 g 100 L^{-1} concentration of Pro-Ca to Empire apple reduced the weight of the fruit.

In the both years, it was found that Pro-Ca concentrations were statistically significant on the fruit flesh firmness (P<0.05). It was identified that in the both years the highest concentrations considerably increased the fruit flesh firmness. While the lowest concentration (62.5 g 100 L^{-1}) was identified to have the least firmness, it was in the same group with the control (Figure 2E). Also, SSC and TA in the year 2018 were also found to be significant (P<0.05) (Table 2). In the first year, 125 g 100 L^{-1} and 250 g 100 L^{-1} concentrations increased SSC and TA, and 62.5 g 100 L^{-1} concentration had almost the same values with the control group. In the both years application concentrations on pH had no difference from control (Table 2). In the 'Smith' pear, Pro-Ca had no effect on SSC, TA, pH and firmness (Carra et al., 2017). Moreover, Çetinbaş et al. (2015) reported that in 'Starkrimson' apple, Pro-Ca had no negative or positive effect on these fruit quality. As chemical pruning, experiments were carried out with different gibberellin biosynthesis inhibitors, which have the same mechanism of action as Pro-Ca. Mefluidide (1000-2000 ppm) application in Camden peach was decreases the growth but decreases the fruit yield in the following year, Dikegulac (1000-1500 ppm) application decreased the growth but didn't not affect the fruit yield, Mepiquat chloride decreased the growth in the application year and flower bud formation in the following year, It was stated that PP528 (400 ppm) application was not effective on growth (Coston and Gambrell, 1983). Bilginer et al. (1995) stated that Paclobutrazol applied from the leaf is more effective on vegetative growth than Redhaven variety in Cardinal and Glohaven varieties, and emphasized that it decreased the shoot length per fruit eye and did not significantly affect the fruit quality. Pro-Ca applications we used in our study, on the other hand, were determined to reduce shoot development, increase fruit yield and not negatively affect fruit quality. Indeed, Prohexadione-Ca is known to interfere with the 3-ß hydroxylation of GA_{20} to GA_1. The net effect is a reduction in immobile, biologically active GA_1 and an increase in the levels of mobile, but inactive GA20 (Evans et al., 1999; Graebe, 1987).

Pro-Ca, which is used in appropriate doses according to the age and development of the tree, has a negative effect on the vegetative development, unlike the chemicals used in the past and started to be abandoned, it breaks down in a short time such as 4-5 weeks. Thus, it is called an environmentally friendly chemical due to its ease and speed. Pro-Ca, which performs an agropetal (bottom-up) transport within the plant, thus does not affect other organs other than the vegetative component applied. Pro-Ca is fully absorbed by the plant within 8 hours after application to the leaves (Evans et al., 1997).

When Pro-Ca effect on fruit color, one of the most important quality parameters of the fruit peach, was assessed; L*, a*, b*, C* and h° values were statistically significant in 2018 (P<0.05), and insignificant in 2019 (Figure 3A, B, C, D, E). Brightness (L*) and yellowness (b*) were reduced by the applications in 2018. The control group fruits had the highest L* and b* values (Figure 3A, C). a* values meaning there was more forming of the color red increased with the Pro-Ca. 62.5 g 100 L^{-1} concentration realized with the highest a* (29.27) value. (Figure 3B). C* value indicating color density increased with Pro-Ca, and h° value indicating color reduced (Figure 3D, E). As the C* color indicates the color density, the application with the densest color was acquired through the use of 125 g 100 L^{-1} concentration (Figure 3E). In 2019, with Pro-Ca application color values of the fruits ranged in the almost the same values with the fruits in the control group (Figure 2A, B, C, D, E). In the observational color assessment, no statistical difference was found in the first year, however, in the following year Pro-Ca was found to be statistically significant (P<0.05) (Figure 2F). In the both years, observational color values increased with the applications, and the highest percentage of color

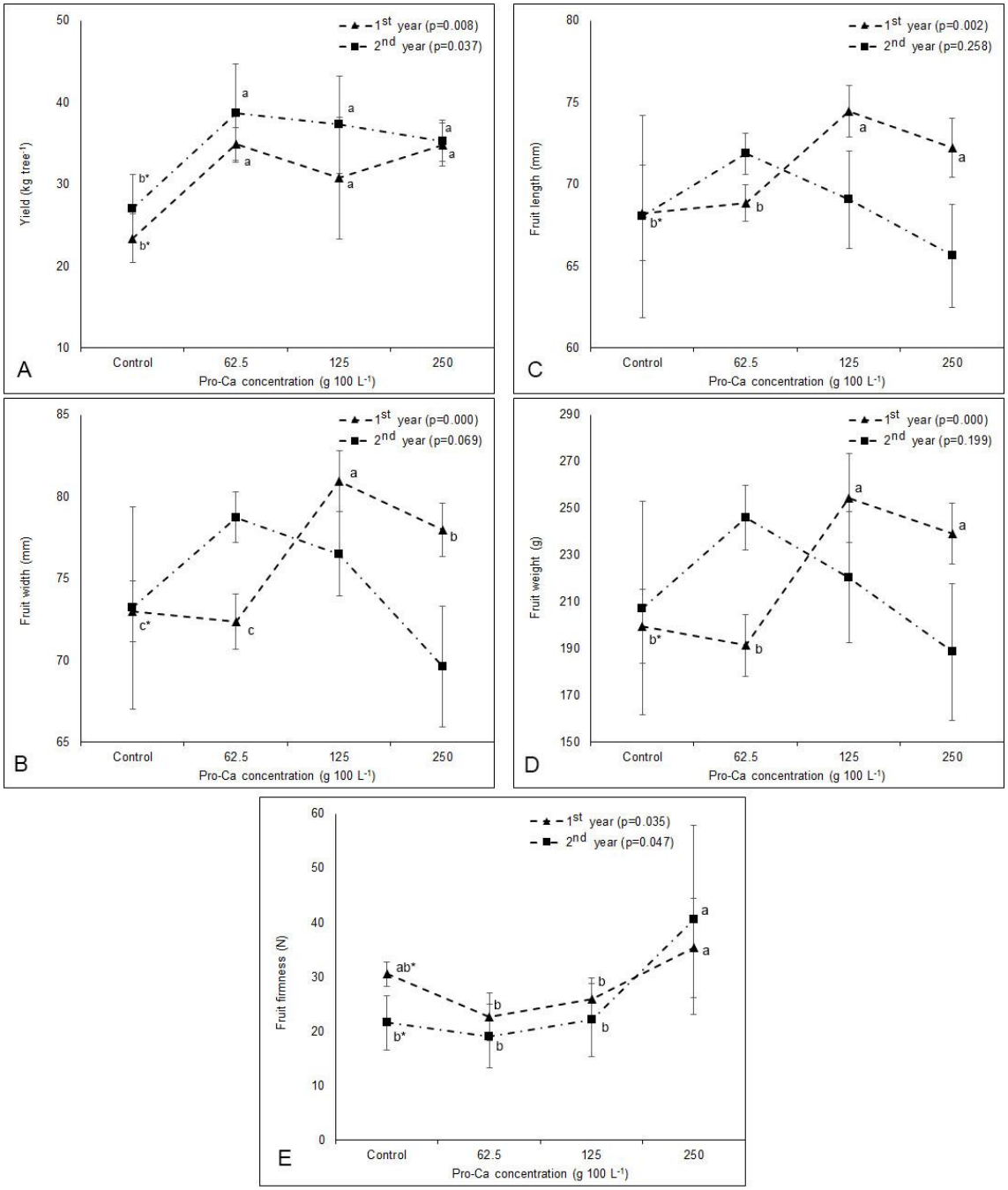

Figure 2. Pro-Ca effects on yield, fruit width, length, weight and fruit firmness in Monroe/GFF 677 (2018 and 2019). Data were mean ± standard deviation. Different letters indicate statistically significant differences among the treatments (LSD, P<0.05).

Table 2. Effects of Pro-Ca on soluble solid content (SSC), titratable acidity (TA) and pH of Monroe/GFF 677.

Pro-Ca concentrations (g 100 L^{-1})	SSC (%)		TA (%)		pH	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
Control	11.70±0.36b*	14.28±0.65	0.46±0.06c*	0.49±0.12	3.59±0.11	3.57±0.11
62.5	11.78±0.35b	14.63±0.78	0.51±0.05bc	0.55±0.15	3.45±0.06	3.63±0.05
125	13.75±0.53a	14.08±1.31	0.58±0.02ab	0.47±0.09	3.57±0.21	3.55±0.10
250	13.00±0.94a	13.90±1.08	0.64±0.12a	0.54±0.07	3.22±0.37	3.57±0.08
P values	0.001	0.703	0.021	0.542	0.134	0.622

Data were mean ± standard deviation.
Different letters indicate statistically significant differences among the treatments (LSD, P<0.05).

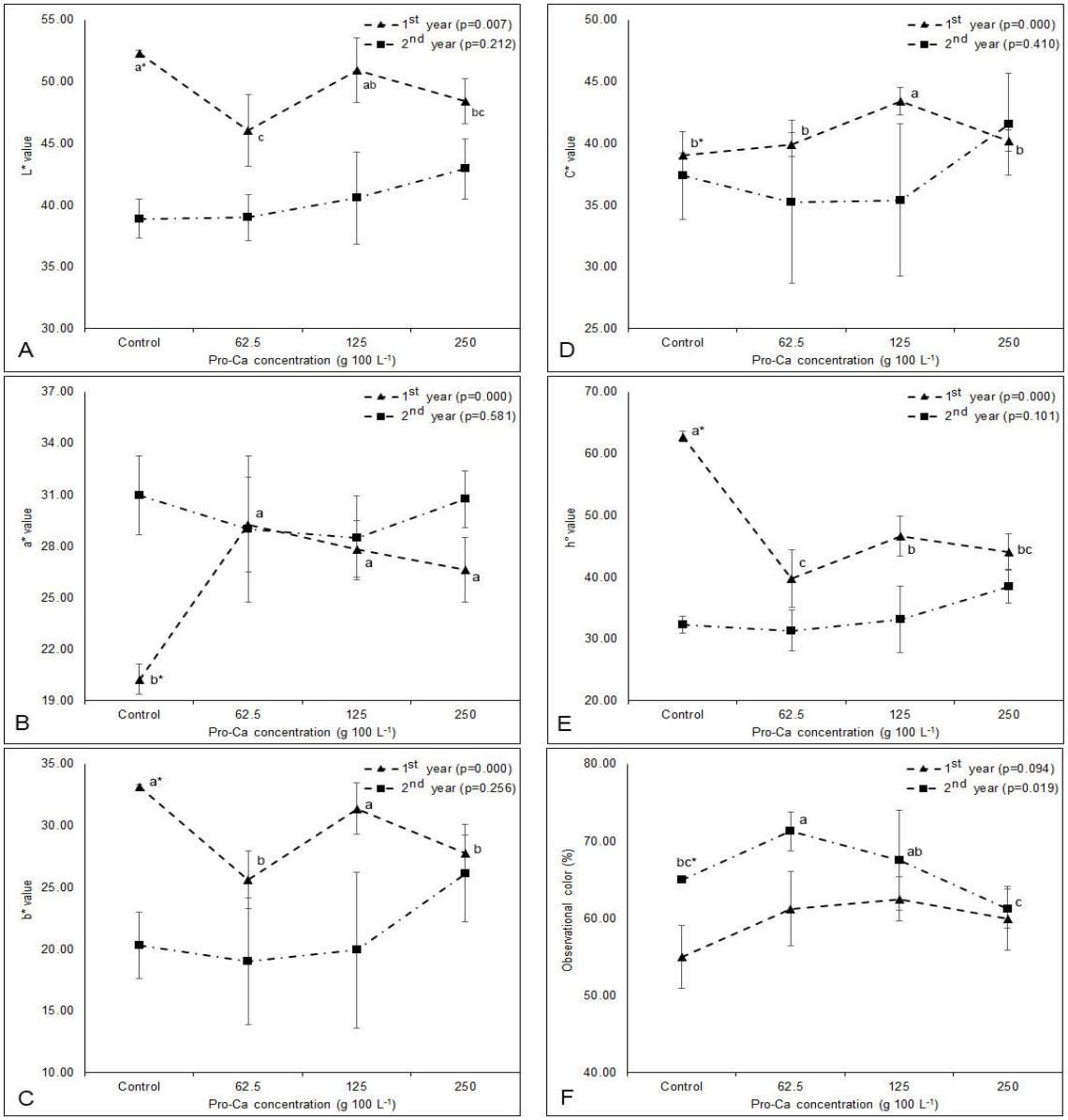

Figure 3. Effects of Pro-Ca on observational color values, L*, a*, b*, C*, h° in the Monroe/GFF 677 (2018 and 2019). Data were mean ± standard deviation. Different letters indicate statistically significant differences among the treatments (LSD, P<0.05).

formation was acquired with the Pro-Ca concentrations 62.5 g 100 L^{-1} and 125 g 100 L^{-1} (Figure 2F). Mata et al. (2006) reported that Pro-Ca had no effect on yield and various fruit quality parameters, but only in 'Fuji', a late season cultivar, exhibited a larger percentage of the red. Hence, in current study, Pro-Ca applications were effective on red color formation in the 'Monroe', a late season cultivar.

4. Conclusions

Pro-Ca had no negative effect on yield and fruit quality, on the contrary 125 and 250 g 100 L^{-1} Pro-Ca exhibited positive results in important fruit quality such as fruit weight and color. Vegetative shoot development drastically reduced, and also average shoot length compared to the control group reduced by 28 to 32%. Pro-Ca increased 40 to 80% the average number of nodes with 250 g 100 L^{-1} concentration compared to the control group. With all the results, Pro-Ca applications had positive effects on the vegetative shoot development control and fruit quality in the Monroe/GF677. 125 and/or 250 g 100 L^{-1} Pro-Ca was found to be more effective than the others.

This study is only a research to determine the Pro applications and their appropriate doses. Especially in the increasingly high density planting peach growing in the world and in Turkey, in the direction of reducing costs and shortening the time of pruning, it is considered to provide additional benefits.

As an alternative to mechanical pruning, chemical pruning can be recommended to apply 125 and / or 250 g / 100 Pro-Ca to peach trees after the shoots are 5 cm and 3 weeks later (2 times).

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RESEARCH PAPER

HORTICULTURAL STUDIES

Wood Charcoal and Ash to Maintain Seed Quality during Storage for Vegetable Seeds

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Abstract

This research was conducted to investigate the efficacy of charcoal and ash in maintaining the quality of lettuce, cabbage, onion, pepper and carrot seeds during storage. The seeds were stored over charcoal and ash at a seed/material ratio of 1:1 and stored at room temperature (20°C) and at 35°C for 24 months. The control seeds were dried to between 5-6% seed moisture content and stored in hermetic packets at -20°C. Samples were collected from storage after 6, 12, 18, and 24 months and subjected to a germination test and ageing test at 45°C for 72 hours using 72% saturated sodium chloride (NaCl) solution. There was no difference between the seed viability for the controls and treatments after 6-12 months at 20 and 35°C between the two storage methods. However, seed quality declined at 18 months and 24 months. This was seen in lettuce, onion and carrot seeds more than pepper and cabbage at both storage temperatures. Similarly, seed vigour also reduced with extended storage to 18 and 24 months. Results showed ash and charcoal can be used to maintain seed quality over 12 months even at 35°C.

1. Introduction

The storage of high-quality seeds in controlled environments is important to ensure the availability of seeds to farmers for commercial and genepool conservation purposes. The seed moisture content and temperature play central roles in the maintenance of seed viability during storage (Roberts and Ellis, 1988; van Treuren et al., 2013; Hanson and Ellis, 2020). In tropical and sub-tropical environments, relative humidity and temperature often go up to 75% and 35°C during storage. When seeds are stored in non-hermetic media seeds absorb excess moisture beyond the safe level for optimum storage due to their hygroscopic nature (Dickie et al., 1990). Such environments also induce the proliferation of storage pathogens. Therefore, the ideal storage temperature and relative humidity for commercial seed storage is about 15-20°C and 30-45% relative humidity for medium-term periods (6-18 months) (Demir and Ozcoban, 2007). However, maintaining such storage conditions is not easy in less developed countries due to high construction and electricity expenses. In this case, alternative storage structures may involve combinations of traditionally-available materials such as salt, lime, ash, charcoal, neem leaves, cow dung etc. to keep seeds dry during storage.

Wood charcoal and ash are cheap, easily available and affordable materials. Due to their hygroscopic nature, they can be used to maintain low seed moisture and prolong the shelf life of seeds (Karthikeyan et al., 2009; Oguntade and Adekunle, 2009; Oyekale et al., 2014; Ashok and Gowda, 2017). Wood charcoal (Karthikeyan et al., 2009; Oyekale et al., 2014; Ashok and Gowda, 2017) and wood-ash were used to keep seeds at low moisture content (Oguntade and Adekule,

2009) and to deter without to the deleterious effects of storage pests (Wolfson et al. 1991; Gumaa and Elamin, 2015) during storage.

This work was designed to test the effect of charcoal and ash on quality during storage for cabbage, carrot, lettuce, pepper, and onion seeds at two different temperatures.

2. Material and Method

Seeds of onion (*Allium cepa* L. cv. Bereket), lettuce (*Lactuca sativa* L. cv. Yedikule), cabbage (*Brassica olarecea* var. Capitata cv. Yalova-1), carrot (*Daucus carota* L. Maestro F1) and pepper (*Capsicum annum* L. cv. Yağlık-28) were purchased from seed companies.

The seed moisture content was determined according to ISTA (2006) rules.

2.1. Storage of control seeds

The control seeds in each species were stored at -20°C with 5-6% seed moisture. These are the conditions in the seed gene bank for long-term storage. Seeds were dehydrated to 5-6% moisture content prior to storage. The seeds were weighed and dehydrated over silica gel in closed plastic boxes at the ratio of 1:5 at room temperature. The silica gel was regenerated every 48 hours at 105°C for 4 hours in a high temperature oven (Ellis et al., 1990) to dry the excess moisture absorbed from the seeds.

During the course of drying, the seeds were weighed twice a day to determine the decline in moisture content and determine the period when the seed moisture content will drop to 5-6% (Demir and Ozcoban, 2007). The formula below was used to calculate the seed moisture content during the course of dehydration.

$$\text{Seed m. c. (g)} = \frac{\text{Initial weight} \times (100 - \text{initial seed m. c.})}{(100 - \text{final seed m. c. \%})}$$

Following seed dehydration to the desired moisture content, they were placed into aluminium foil packets. Four packets were prepared for each species and seven hundred seeds were placed in each packet. Then packets were placed in -20°C. One sample was removed from storage after 6, 12, 18 and 24 months. Of seeds in each packet, 200 were used to conduct the germination test, 200 for the vigour test and the remaining 300 seeds were used to determine the seed moisture content.

2.2. Seed storage over wood-charcoal and wood-ash

Wood charcoal was collected from local charcoal vendors and then ground and sieved into fine forms. The charcoal powder was then dehydrated at 105°C for 24 hours and placed into tightly-closed plastic bottles at room temperature before use. The wood ash (oak ash) was collected from a kebab restaurant and dehydrated to the same values as the charcoal. The dried ash and charcoal were placed in plastic aging boxes (38 × 10 × 15 cm) and the seeds were placed in meshed cloth bags for ease of handling. The material to seed ratio in the storage was 1:1 (w/w). Twenty plastic boxes (2 material × 5 species × 2 temperatures) were prepared with 10 at 35±3°C and the other 10 were stored at 20±3°C. Germination test and accelerated ageing tests were conducted on each sample taken from storage after 6, 12, 18 and 24 months for each species, drying method and storage temperature. The changes in seed moisture after every storage period for each sample were also calculated.

2.3. Germination test

Four replicates of fifty (4 × 50) seeds were used to determine germination percentage (GP). Prior to setting up the germination test, seeds were rehydrated for 24 hours at 100% relative humidity to eliminate potential negative impacts of imbibition damage on the seeds since they were very dry (Ellis et al., 1988).

The seeds were sown between moist germination papers with two papers below and one on top and placed in a germination chamber for 7 days for lettuce, 10 days for cabbage, 12 days for onion, and 14 days for carrot and pepper (ISTA 2006). At the end of the stipulated germination duration (as stated above), normal seedlings were evaluated and referred to as the germination percentage.

2.4. Accelerated aging test

To determine the vigour of the seeds after the various storage durations, 200 seeds from each variety were aged over a saturated salt solution for 72-hours duration.

Forty grams of sodium chloride (NaCl, 76% relative humidity) was dissolved in 100 mL of water as described by Jianhua and McDonald (1996) and stirred diligently. Forty millilitres (40 ml) of saturated salt solution were placed in aging boxes (11 × 11 × 5 cm) with a steel wire mesh (10 × 10 × 3 cm) and the seeds were placed on top of the wire mesh.

The aging boxes with seeds in them were placed at room temperature (25°C) for 24 hours in the dark to enable the seed moisture to equilibrate within the lots. The aging boxes were covered with a plastic film to prevent inflow or outflow of moisture and placed in an incubator at 45°C (Jianhua and McDonald, 1996). After 72 hours, the seeds were removed and subjected to a germination test, and normal seedling percentages were evaluated.

Results were analysed by using SPSS programme and the differences between drying methods in each sampling period were compared at 5% level.

3. Results and Discussion

3.1. Effects of storage on germination percentages

Seed germination percentages were lower in control seeds compared to those stored in ash and charcoal for all five species at both temperatures throughout the storage period. However, the difference in each sampling period was not significant. Germination percentages of control seeds declined from 92 to 84% in lettuce, 92 to 87% in cabbage, 98 to 90% in onion, 98 to 87% in pepper, and 90 to 79% in carrot when seeds were stored at 20°C over 24 months (Table 1). The lowest mean germination percentages were seen in carrot at 77%, while the highest was observed in pepper at 85%. Mean germination percentages of seeds varied between 83 and 90% and 84 and 91% in charcoal and ash, respectively (Table 1). Seeds that were stored at -20°C had a range between 85 and 93%. The difference between the four storage time samples of control, charcoal and ash seeds was significant ($p<0.05$) in three cases for cabbage and onion, two for lettuce, one for pepper and none for carrot seeds.

Storage at 35°C reduced seed germination faster than those stored at 20°C. This was especially seen after 24 months of storage. At this sampling time, the lowest values were seen in ash-stored lettuce and cabbage seeds at 61 and 67%, respectively (Table 2). At this temperature, the difference between ash-stored and charcoal-stored seeds and control seeds started to become significant ($P<0.05$) much earlier. The differences for all four samples of lettuce, onion and pepper were significant ($p<0.05$) starting at 12 months and thereafter in carrot and cabbage seeds. The lowest mean germination percentages were seen in lettuce seeds at the final sampling as 72%.

Carrot and cabbage seeds had 75% and 77% mean germination, respectively. Pepper seeds had mean germination of 84% after 24 months of storage. The difference between mean germination percentages of ash and charcoal storage at 35°C and control seeds were mostly about 4-8% and did not extend more than 10%. The fastest germination loss was seen for lettuce which was stored in ash; seed germination declined from 92% before storage to 61% over 24 months.

Seed moisture and temperature are two main factors that affect seed longevity (Demir and Ozcoban, 2007; Hanson and Ellis, 2020). Seed moisture is the key factor to maintain longevity during storage. For small-sized vegetable seeds, such as lettuce, onion, and cabbage, moisture for long-term storage is supposed to be 5-7% (Walters et al., 2005; Demir et al., 2016 a,b; Hay et al., 2019). As seed moisture increases seed longevity decreases (Hong et al., 2005). However, keeping seed moisture low at about 5-7% is not easy in tropical and sub-tropical regions where relative humidity remains high, i.e. 70%. When seeds are stored under non-hermetic (air and water proof) conditions, seeds equilibrate to the high relative humidity and seed moisture increases. This is a common phenomenon when seeds are kept at room temperature in unpacked conditions. When high seed moisture is combined with high temperature during the summer season in sub-tropical or tropical environments, seed longevity is greatly affected (van Treuren et al., 2013; Bradford et al., 2018).

Medium term seed storage until the next production season comprises about 6-18 months.

Table 1. Germination percentage of seeds of five different species stored at -20°C (Control) and over ash and charcoal for 24 months at 20°C.

Species	Treatment	Storage duration (month)					Mean
		0	6	12	18	24	
Lettuce	Charcoal	92	90^{a}	89^{a}	84^{ab}	78^{b}	87
	Ash	92	89^{a}	86^{a}	78^{b}	74^{c}	84
	Control	92	91^{a}	88^{a}	86^{a}	84^{a}	88
	Mean	92	90	88	83	79	
Cabbage	Charcoal	92	91^{a}	89^{b}	84^{b}	80^{b}	87
	Ash	92	90^{a}	89^{b}	86^{b}	76^{c}	87
	Control	92	91^{a}	92^{a}	90^{a}	87^{a}	90
	Mean	92	91	90	87	81	
Onion	Charcoal	98	94^{a}	88^{b}	85^{b}	83^{b}	89
	Ash	98	95^{a}	89^{ab}	84^{b}	80^{b}	89
	Control	98	96^{a}	93^{a}	92^{a}	90^{a}	94
	Mean	98	95	90	87	84	
Pepper	Charcoal	98	93^{b}	89^{a}	87^{a}	85^{a}	90
	Ash	98	93^{b}	90^{a}	88^{a}	84^{a}	91
	Control	98	97^{a}	92^{a}	89^{a}	87^{a}	93
	Mean	98	94	90	88	85	
Carrot	Charcoal	90	89^{a}	84^{a}	80^{a}	74^{a}	83
	Ash	90	89^{a}	85^{a}	80^{a}	79^{a}	84
	Control	90	88^{a}	85^{a}	81^{a}	79^{a}	85
	Mean	90	89	85	80	77	

Means with the same letters in the same sampling period and species were not significant ($p<0.05$)

Table 2. Germination percentage of seeds of five different species stored at -20°C (Control) and over ash and charcoal for 24 months at 35°C.

Species	Treatment	Storage duration (month)					Mean
		0	6	12	18	24	
Lettuce	Charcoal	92	88^{a}	80^{b}	75^{b}	70^{b}	81
	Ash	92	85^{b}	81^{b}	73^{b}	61^{c}	78
	Control	92	90^{a}	88^{a}	86^{a}	84^{a}	88
	Mean	92	87	83	78	72	
Cabbage	Charcoal	92	90^{a}	85^{b}	83^{b}	76^{b}	85
	Ash	92	88^{a}	84^{b}	80^{c}	67^{c}	82
	Control	92	91^{a}	92^{a}	90^{a}	87^{a}	90
	Mean	92	90	87	84	77	
Onion	Charcoal	98	89^{b}	84^{b}	82^{b}	78^{b}	86
	Ash	98	90^{b}	87^{b}	82^{b}	74^{b}	86
	Control	98	96^{a}	94^{a}	92^{a}	89^{a}	94
	Mean	98	93	88	85	80	
Pepper	Charcoal	98	91^{b}	89^{b}	83^{b}	81^{b}	88
	Ash	98	90^{b}	87^{b}	82^{b}	74^{c}	86
	Control	98	96^{a}	94^{a}	92^{a}	90^{a}	94
	Mean	98	92	90	87	84	
Carrot	Charcoal	90	86^{a}	80^{b}	77^{b}	71^{b}	81
	Ash	90	87^{a}	86^{ab}	77^{b}	70^{b}	82
	Control	90	89^{a}	87^{a}	85^{a}	83^{a}	87
	Mean	90	87	84	80	75	

Means with the same letters in the same sampling period and species were not significant ($p<0.05$)

Our experience showed that vegetable species can be stored with 5-7% seed moisture at about 20°C in hermetic conditions (Demir and Ozcoban, 2007; Demir et al., 2016 a, b) over that period. Such conditions are easy to meet in developed countries. However, low-income farmers and countries may not easily provide such conditions due to economic reasons. Under tropical climate conditions, elements of weather such temperature and relative humidity pose challenges to open air seed storage or in-situ or on-farm seed storage techniques which are common in developing countries (Ellis, 1991; Bradford, 2018). Carrot and lettuce seeds stored in paper bags under ambient conditions at 20°C and approximately 50% relative humidity exhibited great decline in seed germination during the storage period. The decline in germination percentage of lettuce seeds was faster (Nagel and Börner, 2010). Use of cheap and easily available materials can be an alternative method to keep seeds dry. There are number of various materials but ash and charcoal are widely used (Karthikeyan et al., 2009; Oyekale et al., 2014; Ashok and Gowda, 2017).

Our results also indicate that both can be used to maintain quality over 12 months in five species (Tables 1 and 2). Some species were found to lose seed quality earlier than others. Lettuce, onion and carrot appear to be more sensitive species than pepper and cabbage. Longevity changed according to species. Seeds of some species are inherently sensitive to longevity (Walters et al., 2005). Charcoal and ash keep seed moisture low during storage (Table 5). Our results are in agreement with the earlier findings that ash and charcoal keep seeds dry and can be used to maintain seed quality (Karthikeyan et al., 2009; Oguntade and Adekule, 2009; Oyekale et al., 2014; Ashok and Gowda, 2017).

3.2. Effects of storage on seed vigour

Seed vigour test results for seeds stored at 20°C indicated that the most resilient species is pepper. In this species, germination after ageing test was 81 and 83% after 24 months, while these values were 61 and 67% in lettuce, 68 and 66% in cabbage, 34 and 56% in onion, and 66% in carrot seeds for ash and charcoal storage, respectively (Table 3). Seed vigour of ash-stored onion seeds drastically reduced to 38 and 34% with storage to 18 and 24 months. Onion seeds lost seed vigour faster than other species. Mean onion seed germination percentage after 24 months was 57%; this value was highest for pepper at 83%. Pepper seeds lost vigour in germination of just about 10% (93-83%) from the beginning until the end of the storage, but this value went up to 17% in carrot, 19% in cabbage and lettuce, and 38% (95-57%) in onion seeds (Table 3).

Storage at 35°C more drastically reduced seed vigour germination. The minimum reduction was seen in the control seeds. Pepper seeds had the highest vigour germination at 77% for mean germination after 24 months of storage (Table 4). Onion and lettuce seeds had the lowest mean values at 45 and 54%, respectively. Carrot and cabbage seeds had 59 and 67% after the same storage period.

Charcoal and ash onion seeds lost vigour by the end of storage. Vigour germination was reduced to 25 and 27%. Such low values were not seen in any other species. In all species, vigour was lost at 35°C storage starting with the first (6 months) or second (12 months) samplings.

Seed vigour is a part of seed quality, which deals with seed emergence performance in field conditions and storability. Occurrence of seed

Table 3. Germination of seeds of five species stored at -20°C (control) and over ash and charcoal at 20°C after ageing test.

Species	Treatment	Storage duration (month) Vigour (%)					Mean
		0	6	12	18	24	
Lettuce	Charcoal	88	87^{b}	81^{b}	77^{a}	67^{b}	80
	Ash	88	86^{b}	77^{c}	70^{b}	61^{c}	76
	Control	88	91^{a}	89^{a}	84^{a}	78^{a}	86
	Mean	88	88	82	77	69	
Cabbage	Charcoal	90	85^{b}	78^{b}	73^{b}	66^{b}	78
	Ash	90	82^{b}	78^{b}	73^{b}	68^{b}	78
	Control	90	89^{a}	85^{a}	83^{a}	78^{a}	85
	Mean	90	85	80	76	71	
Onion	Charcoal	95	83^{b}	74^{b}	66^{b}	56^{b}	75
	Ash	95	84^{b}	67^{b}	38^{c}	34^{c}	64
	Control	95	94^{a}	89^{a}	86^{a}	82^{a}	89
	Mean	95	87	77	63	57	
Pepper	Charcoal	93	93^{a}	85^{b}	85^{a}	83^{a}	88
	Ash	93	93^{a}	88^{a}	86^{a}	81^{a}	88
	Control	93	91^{a}	89^{a}	87^{a}	85^{a}	89
	Mean	93	92	86	86	83	
Carrot	Charcoal	82	77^{a}	74^{a}	69^{a}	66^{a}	74
	Ash	82	77^{a}	72^{a}	69^{a}	66^{a}	73
	Control	82	81^{a}	75^{a}	70^{a}	64^{a}	74
	Mean	82	78	74	69	65	

Means with the same letters in the same sampling period and species were not significant ($p<0.05$)

Table 4**.** Germination of seeds of five species stored at -20°C (control) and over ash and charcoal at 35°C after ageing test.

Species	Treatment	Storage duration (month) Vigour (%)					Mean
		0	6	12	18	24	
Lettuce	Charcoal	88	76^{b}	73^{b}	66^{b}	40^{b}	55
	Ash	88	74^{b}	73^{b}	53^{c}	43^{b}	66
	Control	88	91^{a}	89^{a}	84^{a}	78^{a}	86
	Mean	88	80	78	68	54	
Cabbage	Charcoal	90	79^{a}	71^{c}	62^{c}	60^{b}	72
	Ash	90	78^{a}	75^{b}	70^{b}	62^{b}	75
	Control	90	89^{a}	85^{a}	83^{a}	78^{a}	85
	Mean	90	82	77	72	67	
Onion	Charcoal	95	87^{ab}	65^{b}	40^{b}	25^{b}	62
	Ash	95	83^{b}	61^{b}	46^{b}	27^{b}	62
	Control	95	91^{a}	88^{a}	86^{a}	82^{a}	88
	Mean	95	87	71	57	45	
Pepper	Charcoal	93	88^{b}	85^{b}	79^{b}	73^{b}	83
	Ash	93	89^{b}	83^{ab}	77^{b}	72^{b}	83
	Control	93	91^{a}	88^{a}	86^{a}	85^{a}	89
	Mean	93	89	85	81	77	
Carrot	Charcoal	82	74^{a}	69^{b}	65^{b}	58^{a}	70
	Ash	82	75^{a}	68^{b}	65^{b}	56^{a}	69
	Control	82	81^{a}	76^{a}	70^{a}	64^{a}	75
	Mean	82	77	71	67	59	

Means with the same letters in the same sampling period and species were not significant ($p<0.05$)

vigour in general precedes seed germination. Accelerated ageing is a vigour test commonly used in a wide range of crop seeds (Demir and Mavi, 2007; Guloksuz and Demir, 2012).

Seed vigour values / germination after accelerated ageing test presented in Tables 3 and 4 showed that vigour loss occurs earlier than germination loss and accelerates as storage duration extends. Moreover, storage at higher temperature reduces seed vigour than storage at 20°C. Seed vigour of species was higher in earlier storage periods, i.e. 6 and 12 months.

Our results showed that charcoal and ash storage not only preserves seed germination but also seed vigour and hence field emergence potential. This is in agreement with the findings of Karthikeyan et al. (2009).

3.3. Changes in seed moisture during storage

Seeds stored both charcoal and ash lost seed moisture gradually during the extended storage period. Seed moisture loss was slightly faster at 35°C compared to 20°C. However, the difference

Table 5. Changes in seed moisture contents of stored five vegetable species seeds in ash or charcoal during 24 months at 20°C and 35°C (italic number). Seed moisture in controls was set with silica gel and hermetic storage at -20°C.

Species	Treatment	Storage duration (month)				
		0	6	12	18	24
Lettuce	Charcoal	8.1	7.9/*7.6*	7.8/*7.0*	6.7/*6.4*	5.9/*4.8*
	Ash	8.1	7.8/*7.3*	6.9/*6.6*	6.1/*6.1*	5.2/*5.3*
	Control	6.2	6.2	6.2	6.2	6.2
Cabbage	Charcoal	8.4	7.9/*7.7*	6.5/*6.3*	5.3/*5.4*	5.2/*4.5*
	Ash	8.4	7.6/*7.3*	6.6/*6.1*	6.0/*5.3*	5.2/*5.2*
	Control	6.3	5.9	5.8	5.8	6.1
Onion	Charcoal	10.1	9.3/*9.8*	8.8/*8.3*	8.3/*7.5*	7.3/*6.1*
	Ash	10.1	9.4/*9.4*	8.7/*8.5*	8.1/*7.2*	7.1/*6.3*
	Control	6.0	5.7	5.4	5.3	5.3
Pepper	Charcoal	8.3	7.6/*7.8*	7.0/*6.3*	6.5/*5.5*	6.3/*4.3*
	Ash	8.3	7.3/*7.1*	6.9/*6.4*	6.5/*5.4*	6.2/*4.4*
	Control	6.0	5.2	5.2	5.1	5.1
Carrot	Charcoal	8.1	7.3/*7.6*	7.2/*6.7*	6.6/*6.7*	6.3/*6.0*
	Ash	8.1	7.3/*7.3*	7.2/*7.1*	6.6/*6.3*	6.3/*5.7*
	Control	6.1	5.5	5.5	5.4	5.4

was up to 1% in some species like pepper over 24 months. By the end of 24 months of storage, seed moisture ranged about 5±1% in all species except onion where seeds stored at 20°C had about 7% moisture. The highest initial seed moisture among all species was for onion at 10.1%. Initial seed moisture in the other four species seeds was about 8.1-8.4%. Control of seed moisture content remained between 5 and 6% throughout storage (Table 5).

One of the important issues with use of such materials is the proportion of seed and material. We used 1:1 in our work. This rate was beneficial for drying rice according to Hay et al. (2012). Our earlier experience also showed this proportion was appropriate. However, the use of higher material proportions may cause extreme drying in seeds during long-term storage and very low seed moisture (<5%) which may be deleterious for seed quality through damaged cell structure (Demir and Ozcoban, 2007; Ashok and Gowda, 2017). In our work, seed moisture declined in some of the samples to below 5% during extended storage periods (i.e. 24 months). Pepper and cabbage seed moisture was reduced to about 4.3-4.5% after 24 months of storage at 35°C. We left seeds at 100% relative humidity before germination testing to avoid seed imbibition damage, which is a common procedure in seed gene banks (van Treuren et al., 2013). The control seeds in our study were stored at -20°C with 5-7% seed moisture. This replicates seed gene bank conditions (Hay et al., 2019) without and are supposed to be the ideal conditions for very long-term storage.

Seeds stored with ash and charcoal germinated as well as those of control seeds during earlier stages of storage, i.e. 6 or 12 month (Tables 1 and 2). This was seen in seeds stored at both temperatures (Tables 1 and 2). Storage at 35°C reduced germination faster than for storage at 20°C during extended storage periods of 18 and 24 months (Table 2). This showed the negative effect of high temperature on longevity (Dickie et al., 1990).

4. Conclusion

The result of the present work showed that storage of lettuce, cabbage, pepper, onion and carrot seeds in charcoal and ash kept seeds dry and preserved germination and seed vigour over 12 months at 20 and 35°C. When seed storage was extended to 18 and 24 months seed quality started to decline, particularly at 35°C. This reduction was seen more for seed vigour than germination. Pepper appeared to be the most resilient species and lettuce and onion are the most sensitive ones. In conclusion, charcoal and ash storage has potential as alternative cheap and easy storage methods for low income farmers and small seed production enterprises.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Application of γ-Aminobutyric Acid Treatment Differently Affects Physicochemical Characteristics of Tomato Fruits during Post-harvest Storage

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Abstract

The quality of tomato fruit, from harvest to human consumption, requires a lengthy period for shipping, storing, and marketing. γ-aminobutyric acid (GABA) is a good candidate because it is a natural substance produced by plants to defend themselves against stress conditions. In this study, the effect of post-harvest GABA treatments at 0 (control), 5 mM and, 20 mM on the physical and biochemical properties and the polysaccharide content of tomatoes during 28 days of storage were investigated. Our results indicated that 5 mM of GABA treatment increased firmness and shelf-life by maintaining the integrity of fruits compared to control and 20 mM of GABA treated fruits. The fruits treated with 5 mM of GABA decreased the amount of WSP and the expression of cell wall related genes *Pectate lyase* (*PL*) and *Polygalacturonase* (*PG*). There was not a clear difference in colour index (CI) values among all treated groups at the end of post-harvest storage. Moreover, the tomato fruits treated with 5 mM GABA also showed somewhat less ethylene production, respiration rate and expression level of two ethylene synthesis genes *ACS2* and *ACS4* towards the end of storage. These results suggested that treatment with 5 mM GABA could be a beneficial strategy for maintaining the morphological and biochemical quality of tomato under post-harvest storage conditions.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an important agricultural crop because of its great nutritive and commercial value. It is one of the most widely produced and consumed vegetable crops with over 180 million tonnes of production and a $190.4 billion commercial value (FAO, 2019). Moreover, tomato is a model species for studying processes such as fruit development, fruit ripening and post-harvest storage conditions (Giovannoni, 2007), because of its relatively short life cycle, highquality with a modest reference genome (approximately 950 Mb), stable genetic transformability and availability of different ripening phenotypes (Klee and Giovannoni, 2011; Wang et al., 2018). The tomato has a relatively short post-harvest shelf life, which limits its transportability and marketability due to its rapid softening rate in post-harvest storage conditions. During storage and transportation, ripening proceeds with a colour change from green to red, as well as softening and compositional changes in taste and aroma-related compounds such as organic acids, sugars, and volatiles (Park et al., 2021). Later stages of ripening/softening are usually accompanied by significant changes in texture resulting from the separation of the cell wall and middle lamellae, as well as water loss by transpiration (Seymour et al., 2013; Uluisik et al., 2016). To overcome the problem of accelerated softening, tomato fruits are usually harvested at the mature green or breaker stage (Wang et al. 2018). Alternatively, novel post-harvest treatments have been investigated to delay

tomato fruit ripening and to extend its shelf-life such as application of aminoethoxyvinylglycine (AVG) (Candir et al., 2017), hydrogen sulfide (H_2S) (Zhong et al., 2021) and UV-C (Mansourbahmani et al., 2018). These chemicals and/or technologies control the transpiration, respiration, ripening of fruits by regulating the biochemical changes in fruits, which delay internal ethylene synthesis in fruits and prolong the market availability of fruits.

Reactive oxygen species (ROS) are composed of several free radical-containing molecules that are regularly found in plants (Foyer et al., 2018) which are harmless when at normal levels. Studies have shown that ROS are key regulators that mediates signalling molecules at low concentrations to trigger defensive responses in fruits and developmental processes (Decros et al., 2019). Nevertheless, under stress conditions or during fruit ripening/softening process the level of ROS in fruits may exceed a certain threshold, which can cause irreversible DNA damage and cell death, resulting in senescence and reduced shelf life of fresh fruits (Chen et al., 2019; Lin et al., 2020). During post-harvest storage life in unfavourable conditions may ascribe to triggering higher ROS accumulation accompanying senescence which deteriorates fruit quality (Lin et al., 2018). Therefore, the use of signalling antioxidant γ-aminobutyric acid (GABA) and molecules, which are key for reducing oxidative stress, may be beneficial in delaying senescence along with preserving sensory and nutritional quality of cherry fruits during post-harvest life (Aghdam et al., 2019; Li et al., 2019; Zarei et al., 2020; Niazi et al., 2021). GABA is a ubiquitous non-protein amino acid produced by glutamate decarboxylation and approved for use in food production in the United States and Europe (Yan et al., 2016). Although under normal growing conditions, GABA content of most plants is relatively low, its accumulation in plant tissues is induced after exposure to various stresses (Kinnersley and Turano, 2000). Moreover, exogenous post-harvest treatment of GABA maintained membrane integrity, delayed browning/softening and preserved nutritional quality (Aghdam et al., 2019; Nazoori et al., 2020). In this study, we aimed to investigate the effect of GABA treatment on tomato softening in post-harvest storage. To achieve this goal, pectin fractionation, gene expression of key pectin-degrading enzymes, ethylene and respiration rates of fruits were evaluated in tomato fruits treated with two different GABA concentrations.

2. Materials and Methods

2.1. Plant material and GABA treatment

Tomato fruits (*S. lycopersicum* L. cv. Verty F1) were grown in a glasshouse, Akdeniz University, Antalya, Turkey, at controlled temperatures of 28-33°C during the day and 20-26°C at night, 85% relative humidity and photoperiod of 12 to 13 hours. Fruits of uniform size that were free from physical defects were harvested at the mature green stage and transported to the laboratory within 2 hours. Fifty-fruits were used for each treatment.

Based on previous studies (Kinnersley and Turano, 2000; Deewatthanawong et al., 2010; Shang et al. 2011; Malekzadeh et al., 2014; Soleimani Aghdam et al., 2016), solutions of GABA (Sigma-Aldrich Co.) at a concentration of 5 mM and 20 mM were selected as the most suitable concentrations. All fruits were immersed in GABA solutions for 30 minutes and then air-dried directly at room temperature. Only distilled water was used for the control fruits. All fruits were stored in plastic trays at room temperature (25 ± 2°C) and 60 ± 5% relative humidity and analyzed for 28 days at seven days intervals. Half of the analyzed fruits were immediately frozen in liquid nitrogen, and then keep at -80°C for biochemical analysis and RNA isolation.

2.2. Determination of fruit colour, firmness, and fruit weight loss

Ten fruits were selected for each treatment group to measure fruit colour. The same fruits were used to measure colour of fruits throughout the experiment. Two symmetrical positions around the equator on each fruit were measured with a colour-meter (PCE-CSM 1) and recorded as Hunter's L^*, a^*, and b^* values. The colour measurements of tomato fruits were carried out twice a week at interval of 3 and 4 days. The colour index (CI) values were calculated according to (Nangare et al., 2016). The average of the maximum forces was recorded from the pericarp of five different fruits for each treatment and each week (PCE-PTR 200 penetrometer) to represent fruit firmness at the ripening stages. Fruit weight loss (FWL) was calculated as the percentage difference between the initial (harvested) and at 7-day intervals until 28 days after harvesting (DAH) of the fruits and calculated according to the following equation: FWL (%) = (DAH28/(DAH0-1) × 100. In this process, morphological changes in the fruits were also photographed.

2.3. Determination of respiration rates and ethylene production

The respiration rate of fruits determined with gas chromatography (GC) (Thermo Finnigan Trace GC Ultra, Thermo Electron S.p.A. Strada Rivoltana 20900 Radano, Milan, Italy). For the measurement tomatoes were stored in 2 L gas-tight jars for 1 h at 20ºC. Then a 1 mL gas sample was taken from the head space of jars and injected into GS equipped with a thermal conductivity detector. The results were calculated as mL CO_2 kg^{-1} h^{-1}. Chromatographic conditions of respiration rate measurement were as follows: 80/100 Porapak-N-column, 65°C oven temperature, 100°C detector temperature, 100°C injection temperature,

10 mL min^{-1} helium flow, 20 mL min^{-1} hydrogen flow, 30 mL min^{-1} nitrogen flow and 4 min analysis time.

The ethylene production was determined by sealing five fruits in a 2 L gas-tight jars for 1 h at 20°C. Then a 1 mL of gas sample was taken from the head space of jars and injected into GS equipped with a flame ionization detector. The results were calculated as μL C_2H_4 kg^{-1} h^{-1}. Chromatographic conditions of ethylene production measurement were as follows: 80/100 alumina f-1 column, 90°C oven temperature, 170°C detector temperature, 150°C injection temperature, 25 mL min^{-1} helium flow, 35 mL min^{-1} hydrogen flow, 350 mL min^{-1} nitrogen flow and 2 min analysis time.

2.4. Extraction and fractionation of cell wall components

The production df colourless alcohol-insoluble solids (AIS) and analysis of the cell wall composition were carried out according to the method described above (Lunn et al., 2013). The methodology of Filisetti-Cozzi and Carpita (1991) was followed for quantification of uronic acids and results were expressed as GA (galacturonic acid) mg g^{-1} of AIS. Samples were left at room temperature for 5 minutes and the colour change was read at the wavelength 405 nm and 450 nm with the difference recorded. The absorbance reading at 405 nm was subtracted from that at 450 nm to correct for interference from hexoses. The results were expressed as milligram of GA g^{-1} of AIS.

2.5. Total RNA isolation, and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from finely powdered pericarp of tomato fruits at 0, 7, 14, 21 and 28 DAH intervals using PureLink™ Plant RNA Reagent (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. The RNA concentration was quantified using the NanoDrop (BioTek, Epoch Microplate) and reverse transcribed into cDNA by the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Relative expression of each gene was normalized to that of the endogenous reference gene *elongation factor 1-α* (Pokalsky et al., 1989) The primer sequences for the RT-qPCR are listed in Table 1. The thermal program for PCR was set using the following conditions: 95°C for 5 min, 35 cycles of amplification of 5 s at 95°C, 30 s at 60°C, 30 s at 72°C using the CFX96™ Real-Time System (Bio-Rad). The gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Determination of proline content

The proline content was determined as described by (Wang et al., 2016). Briefly, 1 g of frozen powdered tomato pericarp tissue was homogenized in 1 mL of 3% (v/v) sulfosalicylic acid and centrifuged at 12 000 g for 10 min at 4°C. 400 μL supernatant was then mixed with 400 μL glacial acetic acid and 400 μL acidic ninhydrin reagent and boiled for 30 min; 800 μL toluene was then added to the reaction mixture. The absorbance was recorded at 520 nm. Known proline concentrations were used to prepare standard curves. The results were expressed as μg g^{-1} DW.

2.7 Statistical analyzes

The statistical analyzes were conducted according to completely randomized design with at least five different tomato fruits for fruit firmness and 10 fruits CI measurements. Three different fruits were used for cell wall and gene expression analysis. Data presented are the means ± SD values. Student's *t*- test, at $p < 0.05$ in SPSS 23.0. was performed to analyze the level of significance between the analyzed parameters.

3. Results and Discussion

GABA can accumulate rapidly under biotic and abiotic stress conditions and take part in the defense system against those conditions (Shelp et al., 1999). Accumulation of GABA has been reported in response to low O_2 storage in tomatoes (Deewatthanawong et al., 2010). In our study, tomato samples were harvested at MG stage, treated with two different GABA concentration, and stored for 28 days at room temperature. Phenotypic variations in ripening tomato fruits after treatment with control, 5 mM and 20 mM of GABA are shown (Figure 1). Although all treated and non-treated fruits showed signs of softening and decomposition at 28 DAH, the fruits treated with 5 mM of GABA appeared to have a better texture integrity compared to 20 mM GABA treatment and control fruits (Figure 1).

Table 1. Primer sequences used for RT-qPCR

Gene	Forward sequence	Reverse sequence
SlPG	AAGACTTGGCAGGGAGGATC	TATGGCCACCTTTGTTGCAC
SlPL	GCGATCAGGAGTTAGAACTGG	AATCCCCTTTTGCTTTGGTT
SlACS2	TATGGAGAGTTATTATAAACGA	CTAAGTACATAGACCAGTTGTCA
SlACS4	ATTCACTAGAGGACTTGAAGA	CAAGCTTTATAACTTTATTTGAT
LeEF-1	ACCTTTGCTGAATACCCTCCATTG	CACAGTTCACTTCCCCTTCTTCG

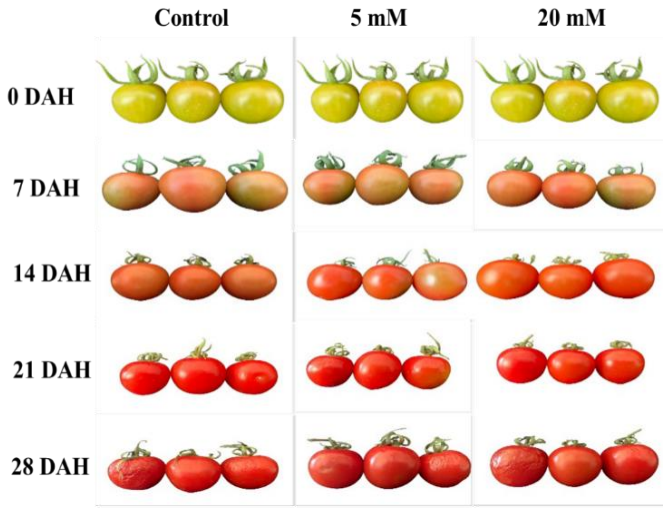

Figure 1. Photographs of tomato fruits treated with GABA taken in seven days intervals (DAH: days after harvesting).

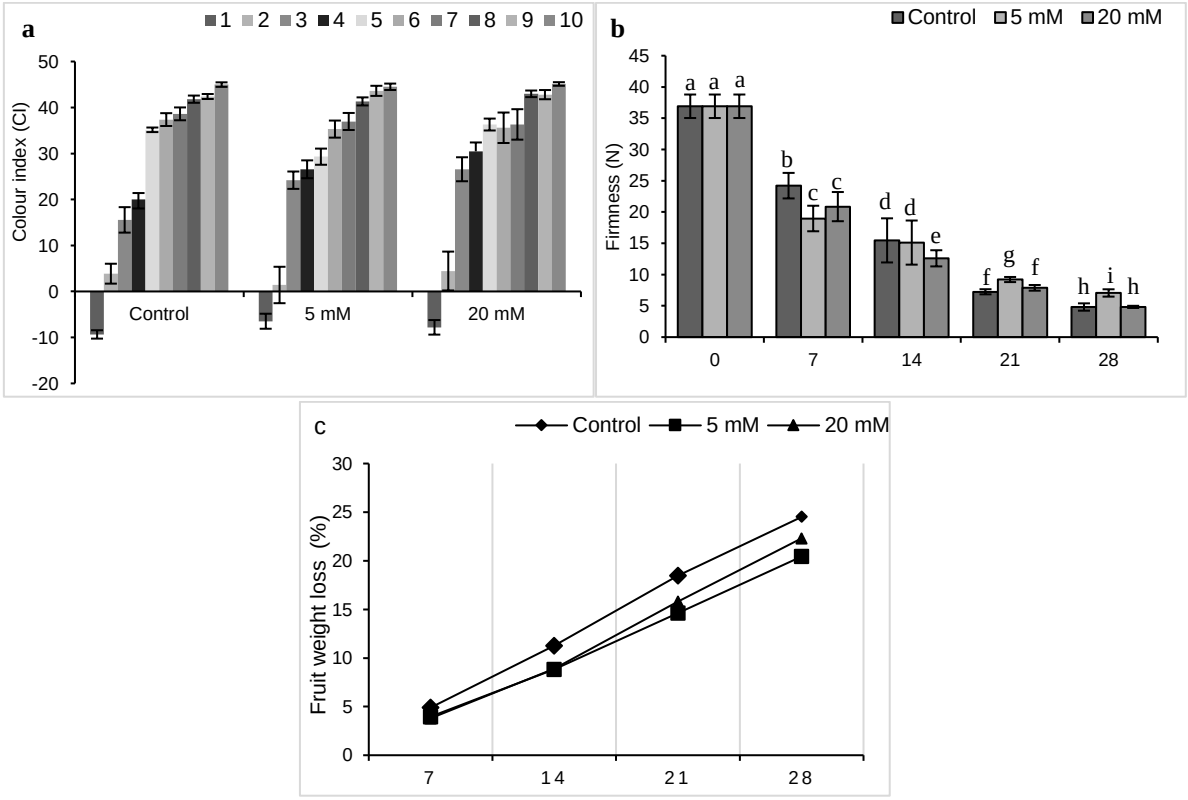

Figure 2. Effect of GABA treatment on CI (a), firmness (b) and Fruit weight loss (FWL) (c) during post-harvest for 28 days. Data are mean ± standard deviation (SD) of ten for CI and five for firmness and FWL independent fruits. Different letters on top of columns indicate statistically significant differences p≤0.05.

The colour index (CI) values of fruits increased sharply in the first four measurements (Figure 2a), which can also be seen 7 DAH and 14 DAH fruits (Figure 1). However, there was no statistical difference in CI values at the end of post-harvest storage ($p > 0.12$).

Firmness is one of the important quality characteristics of fruits which increases their storage potential and provide resistance to diseases and mechanical damage. However, as the ripening progresses, customer acceptance, and marketability of tomato decrease (Distefano et al., 2020). The changes in fruit firmness between control and GABA treated samples stored for 28 days are shown in Figure 2b. The firmness of all fruits treated or non-treated decreased during the storage. Although control fruits at 7 DAH stage were firmer than the GABA-treated fruits, the tomato fruits treated with 5 mM GABA resulted in statistically significant firmness towards the end of storage. In a previous study, Mature-green mango fruits were collected and treated with 50,100- and exogenous GABA. 200mM of GABA treatment led to better preservation of firmness of mango fruits (Rastegar

et al., 2020). In another study, the fruit bodies of mushroom samples were immersed in 0.01, 0.1, 1.0, or 10.0 mM of GABA solution. The results showed that cap browning and weight loss were lower in 0.1 mM GABA treated samples, which retained their firmness better than untreated mushrooms. (Shekari et al., 2021). Based on these results, it can be said that maximum quality and shelf-life in different fruit samples can be achieved by different GABA concentrations.

Evaporation of water from the fruit surface, respiration and metabolic activities cause weight loss during post-harvest storage in fruits. In other words, weight loss in fresh fruit causes fruit softening, ripening, and aging (Bai et al., 2003). The weight loss of the control and GABA treated tomato fruits is shown (Figure 2c). FWL in fruits of all groups increased gradually as ripening progressed. However, the fruit samples treated with 5 mM and 20 mM of GABA presented lower weight loss than that of the control fruits. Post-harvest treatments have minimized the weight loss of mushrooms (Shekari et al., 2021), tomatoes (Makino et al., 2008) and peaches (Shang et al., 2011). The difference in weight loss of fruits is due to the different permeability, chemical properties and applied GABA concentrations.

It is commonly known that loss of firmness and softening in fruits are directly related to changes in cell wall composition especially in pectic polysaccharides. In order to demonstrate the cell wall composition that accounts for differences in fruit firmness, the pericarps of GABA treated, and non-treated fruits were fractionated. A variety of differences in WSP levels at different ripening/softening stages were revealed (Figure 3). The WSP content was the highest in all groups at 14 days of storage. However, the amount of WSP started to decrease in fruits from the 14th day of storage, and the least amount of WSP was obtained in tomatoes treated with 5 mM GABA in all periods. Contrary to the amount of WSP, the amount of Na_2CO_3 (covalently bound pectin) soluble pectin decreased as maturation increased. There was significantly higher amount of Na_2CO_3 soluble pectin in 5 mM GABA treated fruits compared to the control and 20 mM groups.

The cell wall composition change is the result of the coordinate action of cell wall degrading enzymes, including PL and PG (Brummel and Harpster, 2001). In our study, the transcript levels of two main cell wall-related genes are illustrated in Figure 4. At day 7 and 14, the expression of *SlPL* was reduced to half the level of control samples, especially in 14 DAH fruits. 5 mM of GABA treatment slightly downregulated the gene expression of *SlPG* at 21 and 28 DAH stage. In general, as the storage time prolonged, *SlPG* and

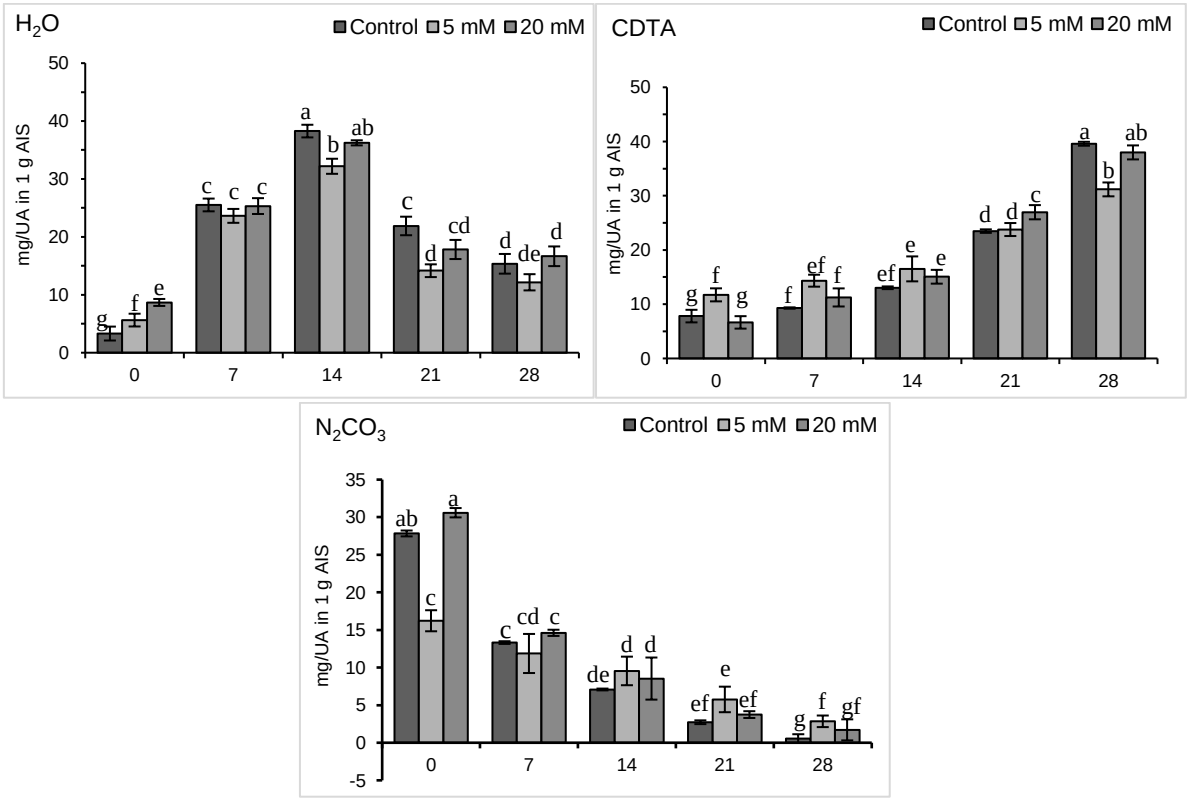

Figure 3. Effect of GABA treatments on WSP, CDTA and Na_2CO_3 soluble pectin contents.
Data are mean ± standard deviation (SD) of three fruits at each stage and treatments.
Different letters indicate statistically significant differences p≤0.05.

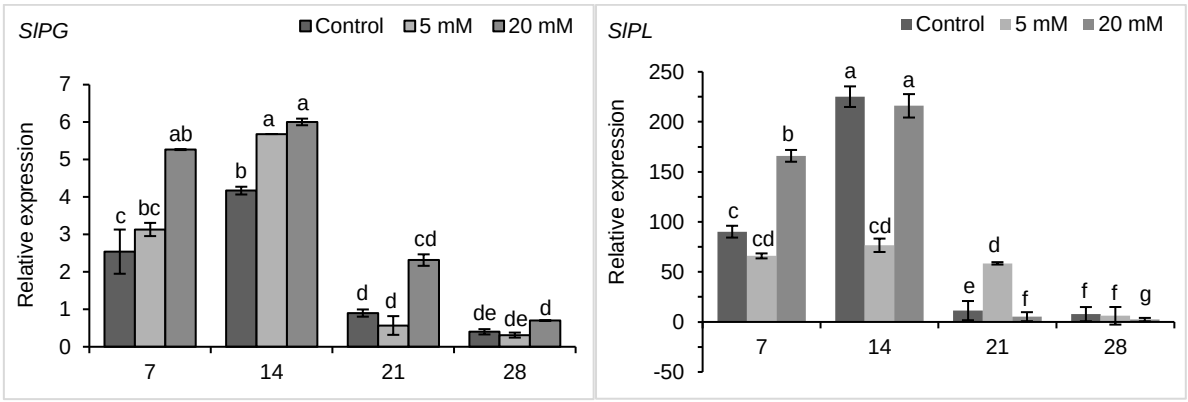

Figure 4. Expression pattern of genes involved in cell wall degradation in fruit ripening process.
The fold change of the genes was normalized compared to control group for each week.
The error bars on each column indicate the ±SD of three biological and two technical replicates.

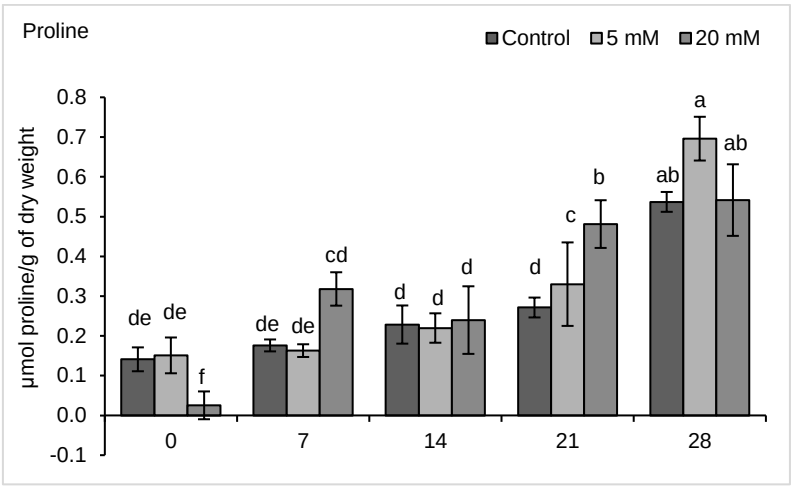

Figure 5. Effect of GABA treatments on proline contents of tomato fruits.
Data are mean ± standard deviation (SD) of three fruits at each stage and treatments.
Different letters indicate statistically significant differences p≤0.05.

SlPL mRNA gradually decreased in all groups. However, a slightly more decrease in 5 mM of GABA treated fruits, could be an increased fruit firmness in this group of fruits. These results indicated that 5 mM of GABA treatment decreased the amount of WSP and expression of cell wall related genes, which delayed softening of tomato fruits under post-harvest storage conditions.

Proline is an important amino acid that has been shown to play an important role in the response of fruits to stress factors as it is an ROS, that can protect cells from damage with its antioxidant effect (Wei et al., 2019). Proline accumulation increased during the exposure of plants to various environmental stresses such as chilling (Gao et al., 2016), low temperature (Mohammadrezakhani et al., 2019) and drought (Antoniou et al., 2017). In our study, a significantly higher proline content was recorded in tomato fruits treated with 5 mM of GABA in 28 DAH fruits ($p \leq 0.014$) (Figure 5). Exogenous GABA treatment enhanced the accumulation of proline and increased resistance against chilling stress (Shang et al., 2011). Another similar study was reported that efficient control of chilling in cherry tomato fruit was associated with enhanced proline accumulation (Zhang et al., 2010). These results indicated that, an optimum concentration of GABA, 5 mM of GABA in here, may be partly related to the increase in proline content and delayed post-harvest softening.

An increase in ethylene production and respiration rate was observed at the beginning of climacteric tomato fruit ripening. Ethylene synthesis in ripening tomato fruit is regulated by 1-aminocyclopropane-1-carboxylate synthase (*ACS*) and 1-aminocyclopropane-1-carboxylate oxidase (*ACO*) gene families (Barry et al., 2000). *ACS2* and *ACS4* mediate the burst of autocatalytic ethylene synthesis, a process typically observed in climacteric ripening (Barry et al., 2000). Ethylene production and respiration rate in GABA treated and control fruits had the similar pattern and the climacteric peak of all fruits emerged at 7 DAH fruits, but the climacteric peaks of the 5 mM GABA treated fruits were clearly lower than those of the control and 20 mM GABA treated fruits (Figure 6a and b). We also detected the relative mRNA levels of the genes related to ethylene biosynthesis. Consistent with the climacteric peak and production of ethylene, the expression levels of *SlACS2* and

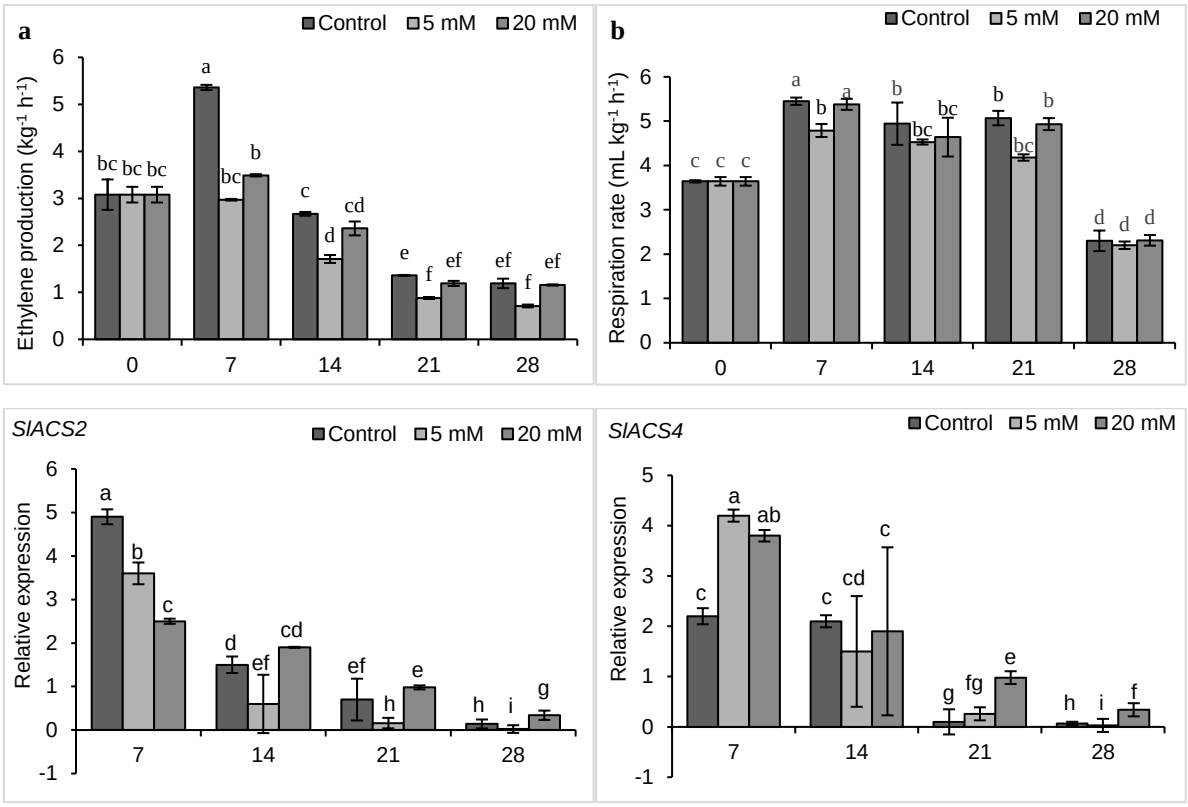

Figure 6. Effect of GABA treatments on respiration rate (a) and ethylene production (b) and ethylene synthesis related genes *SlACS2/4* of tomato fruits during post-harvest storage at room temperature up to 28 days.
Data are mean ± standard deviation (SD) of five fruits at each stage.
Different letters indicate statistically significant differences p≤0.05.

SlACS4 were significantly enhanced at 7 DAH fruits (Figure 6). However, the expression of both genes was significantly suppressed in 5 mM GABA treated fruits in the progress of storage. A value of 10 mM of GABA treatment not only reduced the respiratory rate but also inhibited the ethylene production in apple fruits. Moreover, the expression levels of *MdACO*, *MdACS* and *MdERF* were restricted to varying degrees by 10 mM GABA treatment; this revealed that GABA participates in the regulation of ethylene biosynthesis and signal transduction at the molecular level (Han et al., 2018). It was recently suggested that higher fruit firmness in response to GABA may result directly from GABA treatment or indirectly from lower ethylene biosynthesis or higher level of cell wall hydrolysing enzymes such as PG and PL.

4. Conclusion

The results of the present study indicated that different concentrations of GABA had different effects on tomato quality undur post-harvest storage conditions. A concentration of 20 mM of GABA accelerated fruit softening, fruit weight loss, increased WSP and the expression of cell wall degrading enzymes, suggesting that 20 mM of exogenous GABA treatment is not a suitable concentration for increasing tomato quality parameters in post-harvest storage. The study shed lights on the favourable effects of 5 mM GABA treatment on tomato fruit quality by increasing fruit firmness and shelf life and decreasing the expression of cell wall related genes and dissolution of pectic polysaccharides.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Vacuum Versus Open Air Storage for Pepper (*Capsicum annuum* L.) Seed Longevity with Low Temperature and Seed Moisture Content Over 48 Months

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Abstract

This study was carried out to test the effect of vacuum and open-air storage on seed germination, oil content, and sugar contents of four pepper cultivars. Seeds were stored at 13°C with 35% relative humidity over 48 months in vacuum packets or in perforated cheese cloth in a storage room. Seed samples were examined at 12, 24, 36 and 48 months. Seed germination, oil content and sugar contents were determined. Seed germination declined gradually as storage time extended. Vacuum storage had significantly higher ($P<0.05$) germination than oxygen storage after 48 months of storage for all cultivars. Differences between the two storage methods were not significant for the other samples, except Yaglik in which vacuum storage had higher values from 24 months onwards during storage. Total oil content declined in all cultivars but the decline was faster in seeds stored in the open air. A similar trend was also observed for sugar contents. Seeds stored in the presence of oxygen lost sugar content faster than vacuum-stored seeds. Results indicated that storage with vacuum conditions (no oxygen) extended the longevity of pepper seeds.

1. Introduction

The principal post-harvest environmental factors influencing seed deterioration are temperature, seed moisture content and oxygen pressure (Roberts and Abdalla, 1968; Roberts, 1972; Krishnan et al., 2004). The relationship between seed longevity temperature and seed moisture were well documented and quantified over a wide range of species (Ellis and Roberts, 1980; Walters et al., 2005; Ventura et al., 2008; Kochanek et al., 2010; Sano et al., 2017; Kim, 2018). However, research about the oxygen effect on seed longevity is relatively limited. There was a tendency in seed storage to ignore the role of oxygen and its effect on longevity is considered to be modest in air-dry storage. However, more recent reports indicated that oxygen reduced seed longevity in various crop seeds (Ellis and Hong, 2007; Barzali et al., 2005; Schwember and Bradford, 2011; Gonzales-Benito et al., 2011; Groot et al., 2015). Ellis and Hong (2007) concluded that oxygen is relatively more deleterious to timothy and sesame seeds at lower rather than higher moisture contents. Groot et al. (2015) also confirmed that longevity of lettuce and celery seeds was extended by anoxia in dry storage. Both studies suggested vacuum storage (anoxic) to extend longevity.

Seed storage conditions for commercial purposes by the seed company use air relative humidity of about 35-50% and temperature of about 13-17°C. In these conditions, seeds of each species equilibrate at different seed moistures; for example, pepper seeds equilibrate to about 7.1-7.3% seed moisture. Open seed storage is common in many seed technology practices in which seeds

equilibrate to ambient relative humidity and oxygen is freely available at atmospheric concentrations. This is the case when large amounts of seeds are stored before packaging in air tight packets for sale. For seed germplasm conservation, Groot et al. (2015) suggested that seeds should be stored under dry, cool and low oxygen concentration conditions after harvesting. The objective of this investigation was to determine whether or not seed germination differed between vacuum-sealed containers (hermetic storage/vacuum storage) and open-air seed storage at 35% relative humidity and temperature of 13°C over 48 months.

2. Materials and Methods

2.1. Seed material and storage conditions

Seeds of the pepper (*Capsicum annuum* L.) cultivars Surmeli, K. Dolma, Yaglik and Corbaci were selected for investigation, representing comparatively different fruit shapes and growing habits (Table 1). Seeds were initially equilibrated in a storage room with 35% relative humidity at 13°C over a week. Seeds were weighed daily until no more seed weight changes occurred and seed moisture was determined (wet basis) 103±2°C (ISTA, 2016) and changed between 7.1-7.3% seed moisture. Then, seeds were packed hermetically (vacuumed) or placed in perforated cheese cloth. Eight samples (four vacuum, four open-air) were prepared for each cultivar. Each packet contained 6 g of seeds, except the final sample which only had 2 g of seeds. Seed weight for each sample was measured before storage. Then, 32 samples (4 cultivars × 2 treatments × 4 sampling times) were placed in storage. Seed moisture contents were determined gravimetrically for each sample when taken from storage for germination.

2.2. Germination tests

Seeds were withdrawn from storage at 12, 24, 36 and 48 months. They were then tested for ability to germinate on top of two layers of filter paper (Whatman 42) moistened with 5 ml distilled water in 90 mm Petri dishes at 25°C for 14 days (ISTA, 2016). Radicle emergence was counted after 4, 7 and 14 days during the test. Seedlings were evaluated according to the criterion of normal germination (ISTA, 2016).

2.3. Reduced sugar determination

After adding 5 mL of 15% potassium ferrocyanide and 5 mL of 30% zinc sulphate solution to 2 g powdered pepper seed samples, the mixture was completed to 100 mL with distilled water. The mixture was filtered on filter paper. Then 0.5 mL of the filtrate was taken and 1.5 mL of distilled water and 6 mL of dinitrophenol solution were added. After these processes, the samples were kept in a water bath at 100°C for 6 minutes and then cooled in running water for 3 minutes. The absorbance values of the samples were measured at 600 nm wavelength in a Hitachi brand spectrophotometer. As control in the method, 2 mL of distilled water and 6 ml of dinitrophenol solution were used (Ross, 1959).

2.4. Total oil determination

Dried 2 g pepper seed samples were placed in the extraction cartridge after grinding. The mouth of the cartridge was closed with cotton, preventing the samples from falling out of the cartridge. Cartridges and flasks were placed in the Soxhlet device and extracted in the heating unit (55°C) for 6 hours continuously. After the solvent was completely removed, the balloons were weighed to calculate the percentage of total oil (Cemeroglu, 2010).

2.5. Statistical analysis

The tests were established in accordance with the experimental randomized design. Means for vacuum and open-storage samples in each sampling period were compared using the t test at 0.05% significance. JMP 8.0 statistical package program was used for the analyses.

3. Results and Discussion

Initial normal seed germination of all four cultivars was above 91%. Equilibrated seed moisture content in the storage room resulted in seed moisture content between 7.1 and 7.6%. Mean germination time and seed weight were very similar between cultivars (Table 1). Seed germination percentages gradually declined with storage time in both storage methods. The difference between open-storage and vacuum seeds were not significant (P>0.05) until 36 months of storage for K.

Table 1. Changes in germination (%), equilibrated seed moisture content (%), fruit type, mean germination time (MGT, day) and one thousand seed weight (g) of four pepper cultivars.

Cultivar	Germination (%)		Equilibrated seed moisture content(%)	Fruit type	MGT (day)	1000 seed weight (g)
	Total	Normal				
Surmeli	93	91	7.1	Long-green	5.28	6.41
K.Dolma	92	90	7,2	Bell-shaped	6.05	6.56
Yaglik	95	94	7.3	Capia-red	5.70	6.73
Corbaci	95	93	7.3	Long-green	5.15	6.78

Dolma, Surmeli and Corbaci, but was significant (P<0.05) in the final sampling (48 months). Differences in normal germination percentages between open-storage and vacuum storage were significant (P<0.05) at 24, 36 and 48 months of sampling for the Yaglik cultivar (Figure 1). Final seed germination at the last sampling were 75, 79, 82 and 87 for vacuum storage in the K. Dolma, Surmeli, Yaglik and Corbaci cultivars, respectively, while seeds stored in openly had 67, 69, 68 and 84% germination for these cultivars, respectively. Corbaci cultivar appeared to have the highest germination at the end of the sampling among the four cultivars. Differences between open storage and vacuum storage were greatest at 14% for Yaglik cultivar in the final sampling. This difference was 8, 10 and 3% in K. Dolma, Surmeli and Corbaci cultivars, respectively.

Radicle emergence percentages had differences between vacuum and open-storage methods that were not distinctive, but the difference was more pronounced at the final sampling (Table 2). Corbaci had the highest radicle emergence percentages. The other three cultivars were inferior to this.

Oil contents of pepper cultivars changed between 13.6 and 19.5%, with the lowest for Surmeli and the highest for Yaglik. Total oil content declined gradually with storage time and was lower for open storage than vacuum ones. This was the case for all four cultivars and samples until 36 months. Seeds stored in the open-air lost more than half of their oil content by 36 months of storage. This change was from 13.6 to 6.1%, 17.2 to 8.0%, 19.5 to 8.1% and 17.7 to 6.8% for Surmeli, K. Dolma, Yaglik and Corbaci, respectively. When seeds were

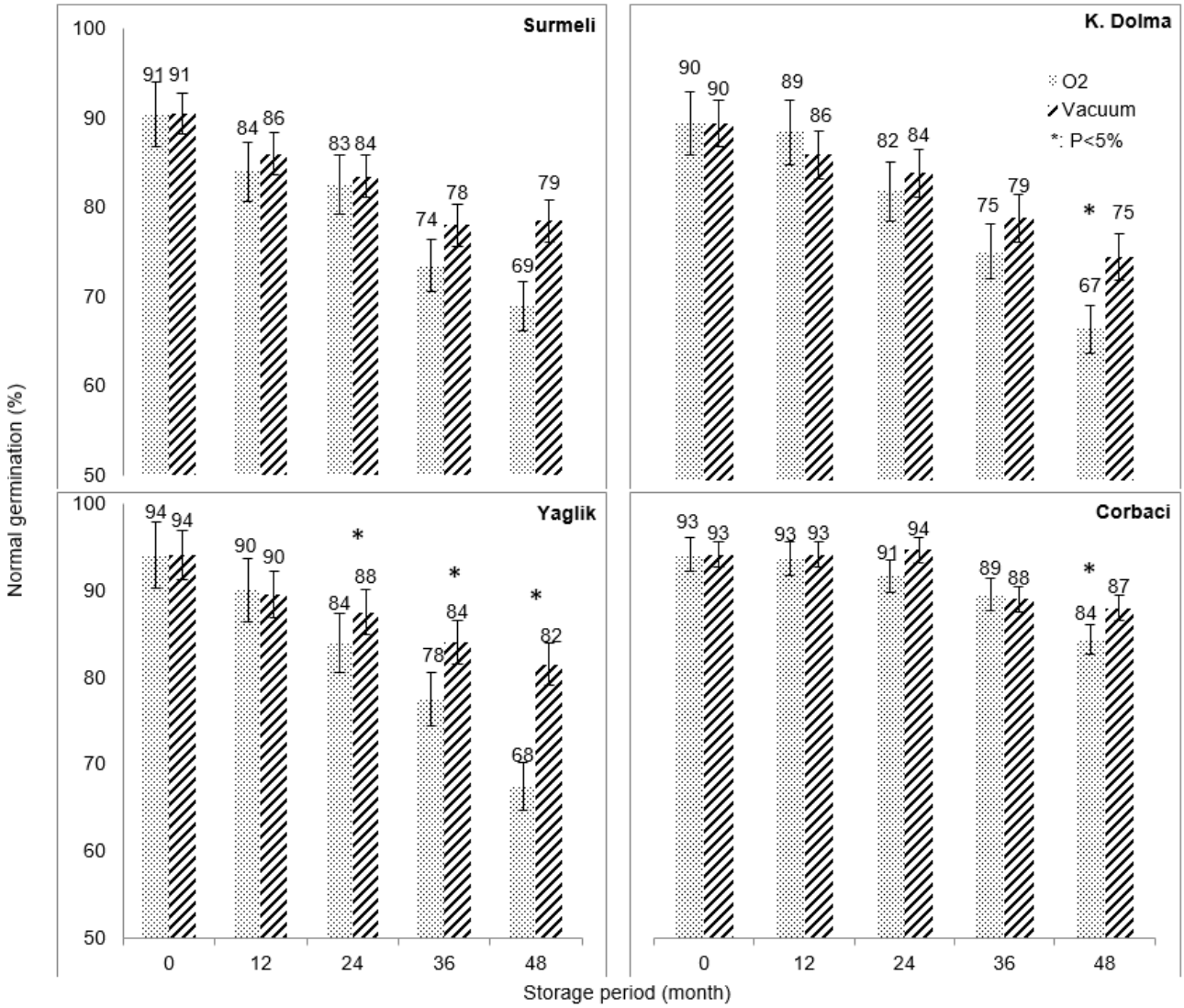

Figure 1. Changes in seed germination of pepper cultivars stored in vacuum and open storage (O_2) conditions during 48 months.
(Bars indicate standard error of mean. Asterisk indicates significant difference at 5% level in each sampling and cultivar).

Table 2. Radicle germination percentages of four cultivars on the fourth day of the germination test.

Cultivar	Storage type	Storage months				
		0	12	24	36	48
Surmeli	Vacuum	62±8.1	63±5.0	57±6.2	58±5.1	58±3.7
	Open-air	62±8.1	53±3.0	53±12.1	58±9.7	41±3.0
K. Dolma	Vacuum	18±0.9	13±1.7	20±2.8	21±5.3	20±3.8
	Open-air	18±0.9	20±3.2	13±4.1	21±7.3	14±4.9
Yaglik	Vacuum	55±6.1	70±3.2	59±.4.0	53±13.9	61±3.3
	Open-air	55±6.1	60±9.2	51±7.3	45±7.8	27±3.3
Corbaci	Vacuum	80±5.4	67±6.2	77±10.7	80±6.5	89±3.0
	Open-air	80±5.4	72±7.5	80±6.2	87±5.0	86±3.0

Table 3. Changes in total seed oil content ±se (%) of four pepper cultivars during 36 months of vacuum or open-storage conditions.

Cultivar	Storage type	Storage months			
		0	12	24	36
Surmeli	Vacuum	13.6±1.3	11.6±2.4	9.8±1.6	8.4±3.4
	Open-air	13.6±1.3	9.5±3.1	7.2±1.7	6.1±2.1
K. Dolma	Vacuum	17.2±2.1	14.4±3.8	12.1±2.6	9.9±1.8
	Open-air	17.2±2.1	12.6±2.4	10.5±1.9	8.0±1.0
Yaglik	Vacuum	19.5±2.0	15.9±2.4	13.1±2.8	10.7±1.7
	Open-air	19.5±2.0	13.2±2.0	9.8±3.2	8.1±2.4
Corbaci	Vacuum	17.7±1.5	13.5±1.8	11.9±1.9	9.5±1.7
	Open-air	17.7±1.5	11.9±3.0	9.1±1.5	6.8±2.1

Table 4. Changes in total sugar content±se (%) of four pepper cultivars during 36 months of vacuum or open-storage conditions

Cultivar	Storage type	Storage months			
		0	12	24	36
Surmeli	Vacuum	7.2±0.8	6.1±0.9	4.3±0.6	3.4±1.0
	Open-air	7.2±0.8	6.4±0.6	2.9±0.3	1.6±0.4
K. Dolma	Vacuum	6.8±1.4	5.1±1.0	3.8±0.8	2.5±0.7
	Open-air	6.8±1.4	5.2±0.4	2.9±.0.4	1.9±0.2
Yaglik	Vacuum	4.6±1.0	3.1±0.8	2.8±0.7	1.4±0.3
	Open-air	4.6±1.0	4.8±1.0	1.7±0.3	0.9±.0.1
Corbaci	Vacuum	5.7±0.8	5.4±1.1	2.9±0.9	2.7±0.9
	Open-air	5.7±0.8	3.0±0.6	1.6±0.2	0.8±0.1

vacuum stored the final oil percentages were 8.4, 9.9, 10.7 and 9.5%, respectively (Table 3).

Sugar content of the cultivars changed between 4.6 and 7.2%, with the lowest for Yağlik and the highest for Sürmeli. Total sugar content reduced with the storage time and reductions were faster in open storage than vacuum storage. By the final sampling, sugar content was 3.4, 2.5, 1.4 and 2.7% for Surmeli, K. Dolma, Yaglik and Corbaci, respectively. When seeds were stored in the open air, these values were 1.6, 1.9, 0.9 and 0.8%, respectively (Table 4).

Results of the present work indicated that vacuum storage was beneficial to pepper seed longevity. Openly stored seeds lose germination earlier than those with vacuum storage. The effect was more prominent in extended samplings, particularly at 48 months of storage. The difference between the two storage methods was significant at the final sampling (48 months) for three cultivars but was significant at 24, 36 and 48 months for the Yaglik cultivar. Our results about the advantages of vacuum storage (oxygen is not available) were reported earlier for various crop seeds (Barzali et al., 2005; Ellis and Hong, 2007; Demir et al., 2009; Schwember and Bradford, 2011; Gonzales-Benito et al., 2011; Soh et al., 2014; Groot et al., 2015; Han et al., 2021) compared to open-air storage ones. The effect of vacuum storage was more prominent at low seed moisture contents than higher one (Ellis and Hong, 2007). Low and high seed moisture contents were not compared in this work. We aimed to obtain results for seed companies. Seeds were equilibrated at 35% relative humidity in both vacuum and open-air conditions where seed moisture ranged between 7.1 and 7.3% (Table 1). The equilibration of pepper seed moisture at this relative humidity was in agreement with Shivhare and Singh (2000). Seed storage temperature was 13°C. In these conditions, medium term storage i.e. 18-36 months is applied at commercial seed production scales. This storage environment is suitable for keeping seed quality for seeds to be sold in the following season. Our results showed that there was an insignificant difference between vacuum and open-storage conditions until 48 months in three out

of four cultivars. However, vacuum storage was favourable compared to open-air in the final samples (Figure 1). This shows that even storage conditions which have favourable presence of oxygen may induce aging during prolonged storage periods.

Differences among the species and cultivars were reported in earlier studies (Ozcoban and Demir, 2002; Basay et al., 2006; Nagel et al., 2009; Panayotov and Aladjadjiyan, 2014; Demir et al., 2020; Yildirim et al., 2020). In our work, Yaglik was the most sensitive to longevity among all cultivars. There may be various reasons for this. One may be the higher oil content of the seeds. Yaglik had the highest oil content which may trigger ageing through lipid peroxidation (Copeland and McDonalds, 1995). Corbaci was the more resilient cultivar. The response of cultivars was clearly seen since the initial seed quality, i.e. normal germination and means germination time of the cultivars, were very similar. Thus, we may assume that pre-storage factors were not influential since all had the same quality at the beginning. Variations in response to oxygen among the cultivars may also relate to the seed coat structures, such as the hard and impermeable coat and presence of diffusion barriers. Cuticles are considered to be the main barrier to oxygen diffusion and permeability increases markedly at temperatures above 35°C (Riederer, 2006). The effect of any differences among the cultivar differences in cuticle composition and permeability in low temperature storage in our study may be more influential.

Various metabolic and structural changes occur during ageing. Lipid peroxidation is a crucial element related in longevity control (Xu et al., 2015). During seed storage, respiration processes utilize the energy kept in the seed, so that a seed which has experienced extended storage usually fails to germinate due to insufficient supply of essential soluble sugars (Eastmond, 2006; Zhou et al., 2019). One of the most commonly observed aberrations reported from aged seeds is disruption of lipid bodies. Owing to the low density of water activity, enzyme activity is not or hardly possible in seeds dried under 40% of relative humidity (Labuza, 1970). As a result, oxygen utilization by aerobic respiration is absent or very low as well. For this reason, oxygen consumption by dry seeds is more likely associated with the creation of superoxide or other ROS molecules and later oxidation of macromolecules, for instance, lipids, phospholipids and DNA. This was noted in various species (Harman and Granett, 1972; Smith, 1980; Salisbury and Roos, 1985). According to Li et al. (2005), the basic mechanism for aging of pepper seeds is related with elevated peroxidation of lipid membranes. When the time of seed storage extends, high amount of peroxidation and oxidation of the lipids in the seed cause a diminished concentration of unsaturated fatty acids and soluble sugars that are created from triacylglycerol (TAG) (Bhattacharya et al., 2015; Zhou et al., 2019). Accordingly in our study, pepper oil content decreased during storage but this was faster in open-air storage than vacuum storage. A similar trend was also seen for sugar content during ageing. Metabolic destruction of sugars was also observed during ageing. The presence of oxygen accelerates the decomposition of both oil and sugar content in pepper seeds during storage. Decreases in oil and sugar content occurred as seed germination was reduced by the extended storage. This cannot be due to the activity of hydrolysing enzymes since seed moisture is low. However, respiration, lipid peroxidation and Maillard reactions are likely to have a combined effect of decomposition of stored materials (Colville and Pritchard, 2019).

4. Conclusion

This study indicated that seed germination after open-storage was inferior to vacuum storage in pepper seeds at low 7.1-7.3% seed moisture and 13°C. The decline in germination was associated with reduced amounts of seed oil and total sugar content. While openly stored seeds have lower oil and sugar content than vacuum ones. Vacuum storage is a preferable practice to achieve maximum germination even in storage with low seed moisture and temperature conditions.

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RESEARCH PAPER

Analysis of the Effect of the COVID-19 Pandemic on the Prices of Basic Food Sold in Traditional Markets: The Case of Jakarta Province, Indonesia

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Abstract

The purpose of this study was to analyze the development and fluctuation of basic food prices in traditional markets in Jakarta before and during the coronavirus disease (COVID-19) pandemic. The study used performance reports from the Indonesian Central Statistical Institute (BPS), National Center for Food Price Information (PHPI), various government agencies, the latest news from reliable online media, and similar studies. The scope of the research focused on the development of basic food prices in traditional markets from January 2019 to May 2021 in Jakarta, Indonesia. The basic food prices discussed in this study were shallots, rice, red chili, and garlic. Results showed that, the impact of the COVID-19 pandemic on the price fluctuations of shallots in the traditional markets of Jakarta was not significant. During the COVID-19 pandemic, rice prices remained stable due to the government's welfare program. The price of red chili in traditional markets tended to decrease due to the COVID-19 outbreak. The COVID-19 pandemic caused fluctuations in garlic prices in traditional markets in Jakarta, and this was because garlic imports from China faced logistical difficulties.

1. Introduction

The World Health Organization has declared a global emergency status for the COVID-19 outbreak. The world is becoming aware of this virus outbreak. Not only alert to the spread of the disease but also alert to the possible impact on the world economy. According to the Managing Director of the International Monetary Fund (IMF), Kristalina Georgieva, the coronavirus outbreak will cause a global economic slowdown in the short term. Global economic growth in early 2020 began to show signs of decline, starting with a decrease in economic growth in developed countries, even in developing countries. This situation is further exacerbated by the COVID-19 pandemic that has experienced almost all countries in the world; the World Trade Organization (WTO) noted that 80 countries had implemented export restrictions (Rahmayani, 2021).

The COVID-19 pandemic has caused significant changes in several sectors in Indonesia, including the food and agriculture sectors. The problem of food availability and fluctuations in the price of basic foodstuffs occurred in various regions; this was due to the implementation of the COVID-19 handling policy in the form of physical distancing and Large-Scale Social Restrictions (PSBB) (Gloria, 2020).

The impact of the COVID-19 pandemic on the agricultural sector covers various aspects, ranging from production, distribution, and consumption of food products. The price of food needs has become erratic. Shallots and garlic are some commodities that experienced an increase in prices; on the other hand, other commodities such as chilies experienced a decrease in selling value. Prices

become erratic, some go up, but some prices go down. Partly this is because demand is falling while supply is steady, so prices are starting to fall (Gloria, 2020). Under Presidential Regulation of the Republic of Indonesia, Number 71 of 2015, rice, chili, and shallots are the basic food needs of the Indonesian people. Basic food needs *are goods related to* the lives of many people with a large scale of the fulfilment of needs and become a supporting factor for the community's welfare. Garlic is the main ingredient for the basic spices of Indonesian cuisine and one of the new sources of economic growth in Indonesia's agricultural development (Rahmawati, 2012). Garlic is one of the horticultural crop commodities whose market demand continues to increase in line with the rapid increase in population, improving economic development and increasing public knowledge about the meaning of nutritional needs. In 2018, Indonesia was listed as the largest importer of garlic in the world. This fact is obtained from the compilation of foreign trade worldwide compiled by United Nations Comtrade (Adharsyah, 2019).

This study aims to analyze the development and fluctuation of basic food prices in traditional markets in Jakarta before and during the COVID-19 pandemic.

1.1. The development of the COVID-19 pandemic in Jakarta

Jakarta is the nation's capital and largest city in Indonesia, with an area of approximately 664.01 km² (ocean: 6 977.5 km²), with a population of 11 100 929. Jakarta is the largest metropolitan city in Southeast Asia and the second in the world. Jakarta is the center of the Indonesian economy. Currently, more than 70% of state money circulates in Jakarta. Jakarta's economy is mainly supported by the trade, services, property, creative industries, and finance sectors (Wikipedia, 2021).

Jakarta is the first province in Indonesia to implement a total lockdown or Large-Scale Social Restrictions (PSBB) because it is the epicenter of the spread of COVID-19 in Indonesia (Ahdira, 2021). Large-Scale Social Restrictions (PSBB) is one of the government's efforts to break the chain of the COVID-19 spread. The implementation of PSBB is regulated in Indonesian Government Regulation Number 21 of 2020, which President Jokowi signed. The implementation of PSBB in Jakarta has been carried out since the beginning of the spread of COVID-19, namely in April 2020, and this PSBB is carried out periodically. Jakarta is the province with the highest number of COVID-19 cases in Indonesia, and the number of people infected with COVID-19 in Jakarta continues to grow from March 2020 to June 2021 (Wijaya, 2020; DKI Jakarta Official Portal, 2021).

Based on the Figure 1, we can see that the number of COVID-19 cases in Jakarta increases from March 15, 2020, to June 8, 2021. The highest number of positively infected people with COVID-19 occurred in January and February 2021, which was 26,029 cases. Then the number of COVID-19 cases decreased in March 2021 after the government imposed a total lockdown. In June 2021, the number of positive people for COVID-19 in Jakarta was 19 096, those who were being treated were 7,856, and those who died were 7,856. At the same time, the number of people who have recovered from COVID-19 is 424 088. Overall, the number of positive COVID-19 cases in Indonesia is 1 927 708, and in Jakarta, 452 295 cases. The death rate from COVID-19 in Indonesia is 2.8%, while it is 1.7% in Jakarta. The cure rate in Indonesia is 91.2%, while in Jakarta, it is 94.1% (Jakarta Smart City, 2021).

Jakarta Governor Anies Baswedan said the increase in COVID-19 cases was due to a decrease in the level of public compliance in implementing health protocols such as wearing masks, maintaining distance, and washing hands.

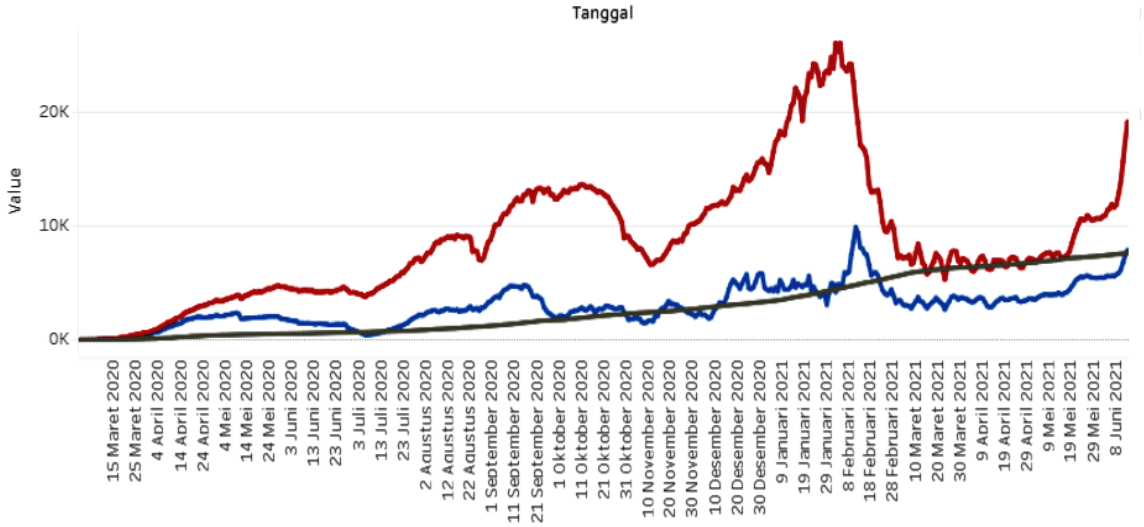

Figure 1. Positive Covid-19 cases in Jakarta (Red line: Positive for Covid-19, Blue line: Under treatment, Black line: Died, Source: Jakarta Smart City, 2021).

Therefore, the Jakarta government has extended the lockdown activities until May 31, 2021, intending to suppress the potential spread of COVID-19 (Wahyudi, 2020). During the PSBB, there were many layoffs (Work termination). Employees are laid off by receiving a reduction in salary/income, receiving money waiting for some time, and other forms that, in principle, reduce people's income. The Indonesian Ministry of Manpower, on June 7, 2020, stated that there were 3.05 million layoffs nationwide until June 2020. This number continues to grow until it is estimated to reach 5.23 million people (Cahyani, 2020). Of course, along with reduced income, people's purchasing power will also decrease. It is believed that after the decline in household income, the demand for basic foodstuffs, including rice, sugar, cooking oil, meat, eggs, also fell. If demand/consumption is low, it will result in low prices, especially if production is constant.

1.2. The impact of the COVID-19 pandemic on traders in traditional markets

Traditional markets are where the activities of sellers and buyers are carried out directly in the form of retail for a temporary or permanent time with a limited level of service. Traditional markets are also meeting centers, folk art activities, information exchange centers and become a unifying social relationship in the community. The COVID-19 case is a global pandemic that has raised concerns from various groups, especially traders in Jakarta's traditional markets. Traders' concerns are increasingly felt when they see the number of positive cases of COVID-19, which continues to increase every day. However, this certainly does not reduce the enthusiasm of the traders to make a living or sell. Because if they wait until this pandemic ends, it will be complicated for traders to meet their household needs (Nuzula, 2021). Traditional market trading activities are still carried out because the community most needs traditional markets to get their daily basic needs. Traders who sell in traditional markets must continue to apply health protocols such as wearing masks, washing hands, and maintaining distance to prevent the spread of COVID-19 (Nuzula, 2021). The impact of the pandemic on traders is a decrease in income due to a lack of buyers. Because during the COVID-19 pandemic, people are required to reduce activities outside the home that affect the economy of traders. The Indonesian Market Traders Association (Ikappi) released the fact that the turnover of traditional market traders in the past month continued to decline by 60 percent during the COVID-19 pandemic (Nuzula, 2021).

1.3. Impact of the COVID-19 pandemic on basic food prices

Regional closures to prevent the spread of the COVID-19 virus have an impact on food logistics disruptions (FAO, 2020a). Several cases show an impact, namely the disruption of marketing access for small farmers to markets so that urban communities find it difficult to access fresh fruits and vegetables, milk, meat, and fish (FAO, 2020b; ACIAR, 2020). Likewise, in Indonesia, the establishment of the PSBB policy creates supply disruptions and delays in food distribution, which can affect food scarcity and rising prices. Moreover, according to Rahma (2020), one week before the PSBB was set in Jakarta, the delivery of rice from the provinces of West Java, Central Java, and East Java to Jakarta had experienced delays, not because of limited food stocks but because of the fear of entering areas classified as the central spread of the virus (Ariani et al., 2020).

Implementing the PSBB regulations for the first time caused panic or fear, especially for people in Jakarta, so panic buying occurred (Fadjarudin, 2020). People who have enough money buy various essential foods and food/drinks in supermarkets in excessive quantities. However, in line with the government's and police's appeal, this only happened for a few days. In the PSBB regulations, food is not one of those that is hampered by its movement because food is a basic need. However, in practice, food distribution experienced a few obstacles due to restrictions on the use of toll roads, ports, airports issued by the Ministry of Transportation through Law Number 25 of 2020. This regulation is specifically related to areas with PSBB status, including regulating a temporary ban (April 24) until May 31, 2020) exit and enter the PSBB area/red zone for land vehicles, trains, ships, and planes users. For logistics/goods, transportation of essential materials and emergency matters are excluded. Then there is a rapid test for people leaving/entering the red zone, Jakarta residents are prohibited from going home, and there are penalties for vehicles that violate; this affects the distribution of food to be less smooth. Food stocks between regions are less evenly distributed because regions experience a food deficit, and some excess experience production. Food distribution constraints are also a result of policy changes from exporting countries trying to save production for domestic needs so that imports of agricultural and food products are delayed or not smooth (Coordinating Ministry for Economic Affairs, 2020).

The PSBB regulations have an impact on food distribution and have an effect on increasing food prices, thus affecting people's income (Celik et al., 2020). Moreover, this is reflected in economic growth performance in the first quarter of 2020, which decreased by 2.97% (BPS, 2020a). This economic contraction affects the narrowing of employment opportunities (decreased working hours and layoffs), impacting income and purchasing power. Around 44.7% of male respondents and 38.6% of female respondents admitted that they experienced a decrease in

income (BPS, 2020b). A decrease in income will undoubtedly affect a decrease in food demand; although the magnitude will vary according to income, group and food function (as basic food, luxury food, or substitute food). The analysis conducted by Suryani et al. (2016) using data from the 2014 National Socio-Economic Survey (conditions before the COVID-19 pandemic) shows the income elasticity for rice, chicken meat, beef, and eggs, fish, shallots, and red chilies is positive. However, the elasticity value at low-income households is smaller than wealthy households. An increase in income will increase food demand, and conversely, a decrease in income will reduce food demand.

An increase in food prices will cause a decrease in demand, but the percentage of the decline depends on the type of food. An increase in prices and a decrease in income due to the COVID-19 pandemic will undoubtedly cause a decrease in food demand. According to researcher from the Center for Strategic and International Studies (CSIS) Department of Economics, Haryo Aswicahyono, people's purchasing power has decreased due to the outbreak of COVID-19 in Indonesia since March 2020 and this has led to relatively low movements in the consumer price index (CPI). The decline in people's purchasing power is inseparable from the decline in people's incomes, especially those with irregular incomes (Ramli and Djummena, 2020). According to the Minister of Finance, Rahma (2020) reported that Indonesia's economic growth in the third and fourth quarters was negative, one of which was caused by a decline in household consumption. The purchasing power of the people lost during the pandemic is estimated at around USD 25.4 billion. This calculation is based on the number of working hours lost due to the PSBB policy (Ariani et al., 2020).

Producers of fresh and processed food must address the decline in demand. For food produced by farmers to be sold at a reasonable price, the Ministry of Agriculture must re-calculate the supply of food originating from domestic production. If it is estimated that the products produced by farmers exceed their needs, some things can be done, namely by asking farmers to plant other commodities or processing food into semi-finished food temporarily. If the demand for chili decreases, the excess chili production is processed into ground chili by farmers (gapoktan) or in collaboration with chili processing business actors who are also partners in marketing the ground chili (Ariani et al., 2020).

2. Materials and Methods

This study uses secondary data obtained from survey results from the Indonesian Central Statistics Agency (BPS), the National Center for Food Price Information (PHPI), performance reports from several government agencies, the latest news from trusted online media, and similar studies. The scope of the research focuses on the development of basic food prices in traditional markets in Jakarta, Indonesia, from January 2019 to May 2021. The basic food prices discussed in this study were shallots, rice, red chilies, and garlic. The discussion was carried out in a qualitative descriptive manner, comparing monthly prices, in the period before the COVID-19 pandemic from January 2019 to February 2020 and during the COVID-19 pandemic from March 2020 to May 2021.

3. Results and Discussion

3.1. Shallot price development

Shallots (*Allium ascalonicum* L.) is one of the basic foods of the Indonesian people following Presidential Regulation no. 71 of 2015. Shallots are Indonesia's leading vegetables that have a significant role and need to be cultivated intensively. Shallots are used as a spice. In addition, shallots are used as traditional medicine (Dewi and Sutrisna, 2016). The monthly price of shallots in 2019, before the COVID-19 pandemic, was relatively stable, with fluctuations between 13% to 47%. The highest price of shallots in 2019 occurred in April, which was 3.14 USD kg^{-1}. Moreover, the lowest price of shallots in 2019 occurred in September, which was USD 1.72 USD kg^{-1} (Figure 2). During the beginning of the COVID-19 pandemic, from March 2020 to June 2020, the price of shallots continued to increase, but from August to September, the price decreased. The highest price of shallots in 2020 occurred in May, which was 4.09 USD kg^{-1}. The lowest price in September was 2.35 USD kg^{-1}. From Figure 2, it can be seen that the price of shallots during the pandemic fluctuated highly and reached the highest price in May 2020. According to the National Food Price Information Center (PHPI), the price of shallots was high from April to June 2020 due to reduced shallot stocks because the planting schedule was postponed due to high rainfall (PHPI, 2021). High rainfall causes many shallots to be damaged, thereby reducing the number of shallot seeds that will be used the following year. In addition, high rainfall causes the productivity of shallots to decrease. Usually in 1 hectare can produce 12 tons of shallots, but 1 hectare can only produce 6-7 tons of shallots due to high rainfall. Shallot production is reduced while demand is high, causing the price of shallots during the pandemic to be high. The price of shallots is stable again from January to May 2021 (Yuniarta and Mahadi, 2020).

3.2. Rice price development

Rice (*Oryza sativa* L.) is a basic food for most Indonesian people. Rice consumption in Indonesia

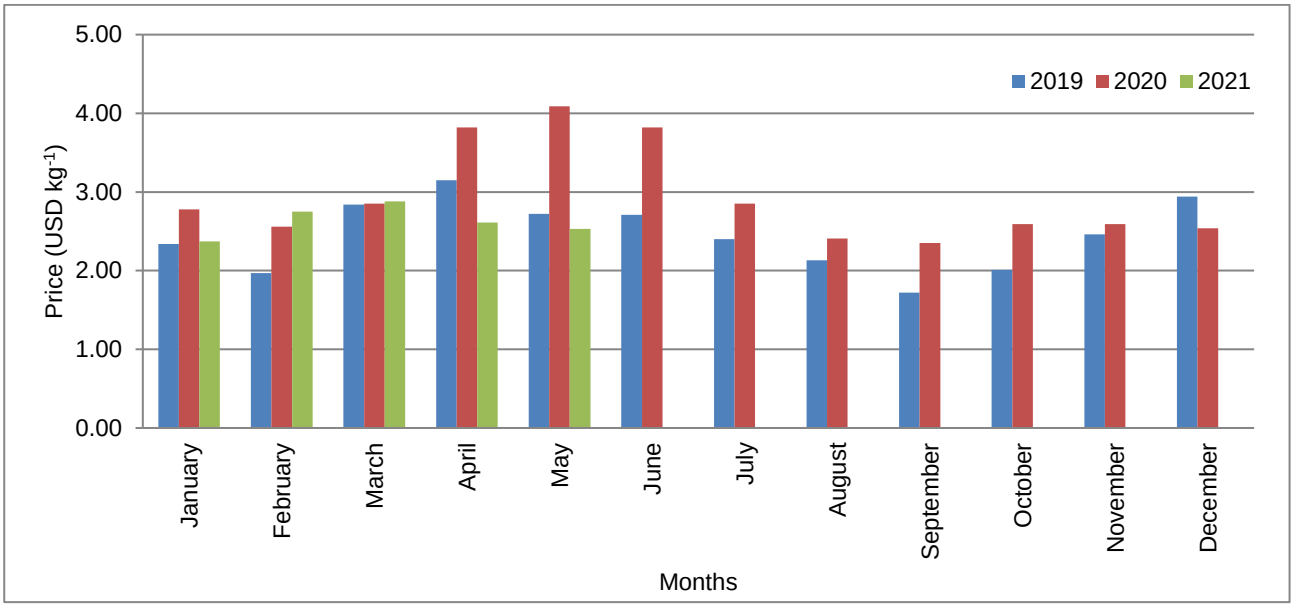

Figure 2. Monthly prices of shallots (USD kg^{-1}) in Jakarta from January 2019-May 2021 (Source: Own calculation).

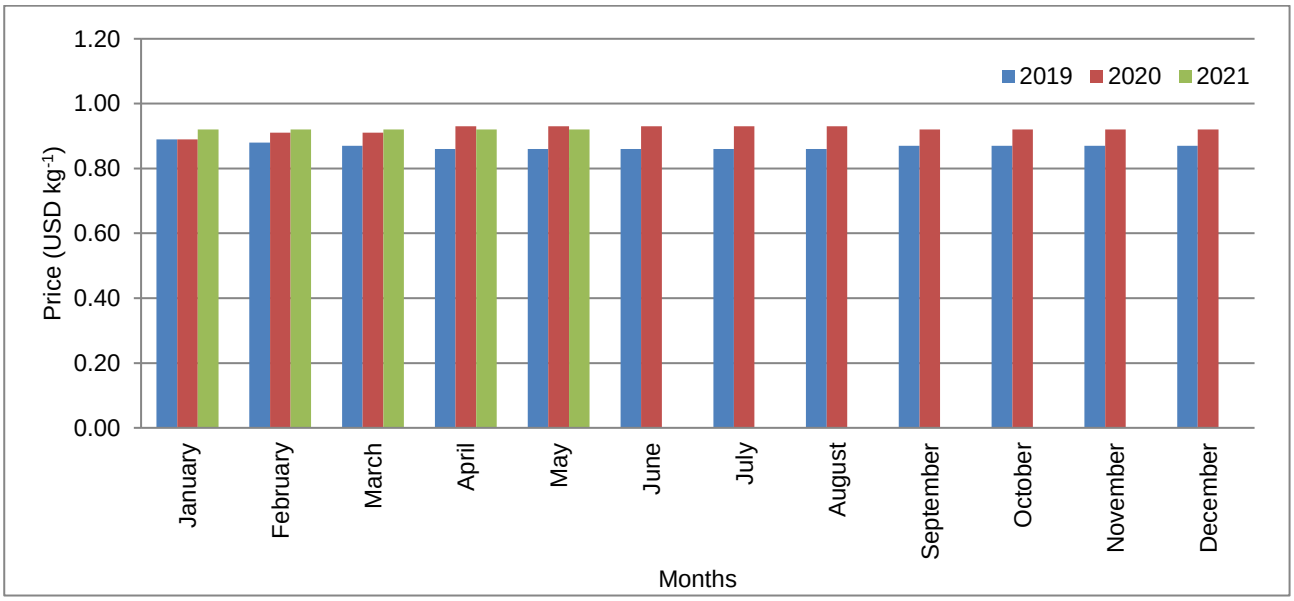

Figure 3. Monthly price of rice (USD kg^{-1}) in Jakarta from January 2019-May 2021 (Source: Own calculation).

is increasing every year along with the increasing population of Indonesia. National rice consumption in 2019 was 94.9 kg $capita^{-1}$ $year^{-1}$. National rice consumption in 2020 will increase by 111.58 kg $capita^{-1}$ $year^{-1}$ (BKP, 2020).

Based on Figure 3, it can be seen that the monthly price of rice in 2019, before the COVID-19 pandemic was relatively stable. During the COVID-19 pandemic, the rice price was also stable from early 2020 to May 2021. Currently, rice is the most stable basic food commodity. Rice prices have been stable during the COVID-19 pandemic due to the government's social assistance program. The government provides social assistance in the form of rice supplies sent to each community's homes in Jakarta. Many people received rice assistance, thus reducing the demand for rice in the market. Rice prices are predicted to be stable until early 2022. This is driven by optimism for a rice surplus in 2020 of 6 million tons. This amount is sufficient to meet the national rice needs in 2021. In addition, in April 2021, the rice supply will increase again as it enters the main harvest. The current condition of rice prices is excellent because there is no price decline at the farmer level and no high price increase at the consumer level (Uly, 2021).

3.3. Red chili price development

Before the COVID-19 pandemic in 2019, the price of red chili (*Capsicum annum* L.) fluctuated every month. The highest price of red chili in 2019 occurred in August, which was 5.39 USD kg^{-1}. Moreover, the lowest price in 2019 occurred in

February, which was 2.03 USD kg^{-1}. Monthly price increase during 2019 between 3% to 58% (Figure 4). The volatility of red chili prices is difficult to control because of consumer preferences who prefer fresh chilies that do not last long in storage compared to processed chilies. The price of red chili is high from June to August 2019 due to fewer farmers growing chilies and planting disturbances due to the dry season. At that time, chili farmers were constrained by land that did not have a sufficient water supply. So that the production volume is not maximized and farmers switch to other crops. From September to December 2019, the price of chili decreased due to abundant production (Andri, 2019).

The price of red chili at the beginning of 2020 increased again, with an increase of 71.5% compared to January 2019. At that time, the rainfall was high, so that the red chili stock was reduced from the farmers (Figure 4). During the COVID-19 pandemic in March 2020, the price of red chili tends to decrease. Red chili prices experienced a sharp decline from April to September. The lowest price of red chili in 2020 occurred in July, which was 1.97 USD kg^{-1}. According to Susilowati and Gunawan (2020) the price of red chili decreased during the COVID-19 pandemic due to the decline in people's income, which caused the demand for red chili to drop drastically. Because during the PSBB (Lockdown), many people lost their jobs and employees were laid off by receiving a reduction in salary/income. Of course, along with reduced income, people's purchasing power will also decrease. Red chili prices have decreased during the COVID-19 pandemic, which is very detrimental to chili farmers. Because the price received is not commensurate with the production costs incurred. In addition, the demand for red chili decreases when farmers are harvesting. From the end of December 2020 until March 2021, red chili prices began to improve. The price of red chili at the consumer level has reached the highest level during the COVID-19 pandemic, 4.60 USD kg^{-1} (Figure 4). Although the price of red chili rose at the consumer level, unfortunately, farmers have not been happy. Due to the price increase from the end of December 2020 to May 2021, it has not covered the losses suffered by farmers due to the deep price decline during the COVID-19 pandemic. The increase in the price of red chili from the end of December 2020 to May 2021 was caused by a lack of stock because several chili plants were attacked by plot disease and fruit rot; this happens every year during December-March 2020 due to high rainfall. Not to mention the contribution of the La Nina phenomenon during October 2020-March 2021, which also contributed to increasing rainfall; when the rainfall is high, chili plants are susceptible to pests and diseases (Thomas, 2021).

3.4. Garlic price development

Garlic (*Allium sativum* L.) is one of the vegetable plants needed by households in Indonesia every day as a cooking spice. Currently, garlic is also one of the new sources of economic growth in agricultural development. Indonesia is China's most significant market share for garlic. Every year Indonesia receives imports of garlic commodities from Jining City, Shandong Province, China. Currently, 90% of garlic sold in the Indonesian market comes from imports (Gunawan and Sayaka, 2020).

Indonesia is only able to produce approximately 4% of the total national demand for garlic every year. This condition is the leading cause of the increase in Indonesian garlic imports (Syafina, 2019).

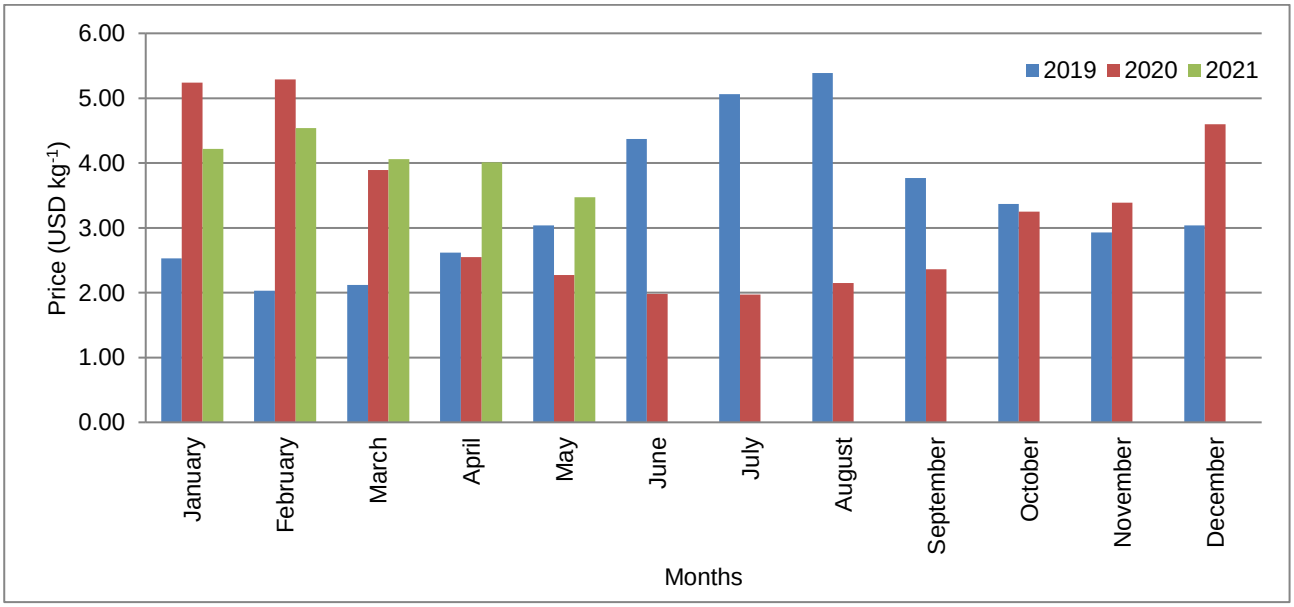

Figure 4. Monthly price of red chili (USD kg^{-1}) in Jakarta from January 2019-May 2021 (Source: Own calculation).

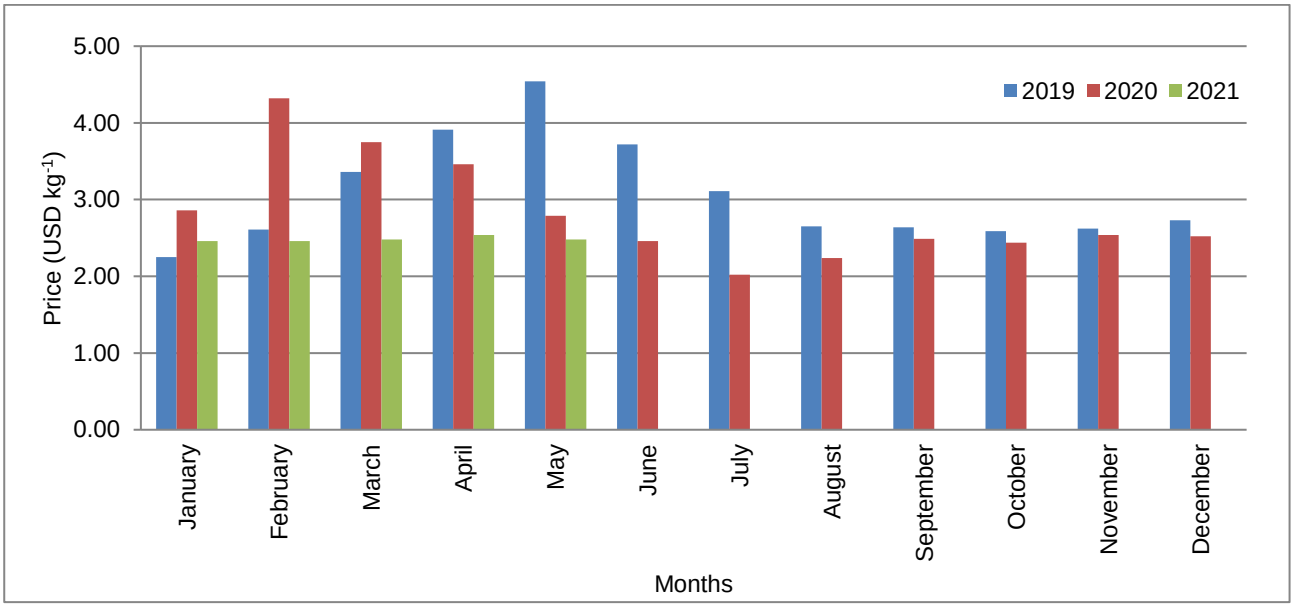

Figure 5. Monthly price of garlic (USD kg^{-1}) in Jakarta from January 2019-May 2021 (Source: Own calculation).

In 2019, before the COVID-19 pandemic, the price of garlic fluctuated every month. The highest price of garlic in 2019 occurred in May, which was 4.54 USD kg^{-1}. The lowest price in 2019 January was 2.25 USD kg^{-1}. The price of garlic was high from April to July 2019 due to the lack of garlic stock (Figure 5) due to delays in distributing imported garlic stocks to traditional markets. There are many imported garlic stocks, but they are late in the distribution process to the market, so prices are high (Widyastuti, 2019). Although it has issued a garlic import permit to maintain the commodity price stability, the government is deemed necessary to monitor the price and circulation of one of these staple commodities. The government must also ensure sufficient stock of garlic.

At the beginning of 2020, the price of garlic rose sharply. In February, the highest price was 4.32 USD kg^{-1} (Figure 5); this happened because the primary source of garlic imports in China was in lockdown, resulting in logistical difficulties to import in early 2020. Based on data from the Central Statistics Agency (BPS), throughout the first semester of 2020, Indonesia imported 284 363 tons of garlic. This figure increased by 141% compared to the same period in the previous year of 117 827 tons (Thomas, 2021). The increase occurred during the coronavirus pandemic. The Indonesian Trade Minister released a new policy that loosened restrictions on garlic imports to contain soaring garlic prices. Therefore, the price of garlic from June 2020 to May 2021 is stable.

Meanwhile, garlic production in Indonesia continued to decline throughout the year. The government should make a program to develop garlic cultivation in Indonesia to not depend on China because the agricultural area in Indonesia is still quite large and has the opportunity to increase garlic productivity. The government needs to provide superior varieties with high yields to increase garlic production. In addition, the government must also raise the enthusiasm of farmers to return to planting garlic.

4. Conclusions

Based on the data that has been researched, the impact of the COVID-19 pandemic is not a significant factor in the price fluctuations of shallots in Jakarta's traditional markets. The main factor causing fluctuations in the price of shallots is the reduced stock of shallots; this is due to the postponement of the planting schedule due to high rainfall. High rainfall caused many damaged shallots. Eventually, the seeds that were deviated by farmers to be planted the following year were reduced. In addition, high rainfall causes the productivity of shallots to decrease.

Farmers must use the rain shelter method in planting shallots when rainfall is high. The rain shelter method can overcome fusarium disease so that shallot plants do not get moldy and do not rot and reduce labor costs when caring for onion plants in the rainy season (Budi, 2018). In addition, the cost of sanitation is cheaper and economical. Other benefits include supporting the application of environmentally friendly cultivation because it reduces the use of pesticides in the field, ensures a successful harvest during the rainy season, maintains humidity, fertilizers in the land are not easily lost due to rain, and cultivation will become more economical and efficient.

It was found that the price of rice during the COVID-19 pandemic has remained stable. Rice prices have been stable during the COVID-19 pandemic due to the government's social assistance program. The government provides social assistance in rice supplies sent to each community's homes in Jakarta. Many people

received rice assistance, thus reducing the demand for rice in the market. Rice prices are predicted to be stable until early 2022. This is driven by optimism for a rice surplus in 2020 of 6 million tons. This amount is sufficient to meet the national rice needs in 2021. In addition, in April 2021, the supply of rice will increase again as it enters the main harvest. The current condition of rice prices is excellent because there is no price decline at the farmer level, and there is no high price increase at the consumer level.

Results showed that the price of red chili in traditional markets tends to decrease due to the COVID-19 pandemic. The price of red chili decreased during the COVID-19 pandemic due to the decline in people's income, which caused the demand for chili to drop drastically. Because during the PSBB (lockdown), many people lost their jobs, and employees were laid off by receiving a reduction in salary/income. Of course, along with reduced income, people's purchasing power will also decrease. Chili prices have decreased during the COVID-19 pandemic, which is very detrimental to chili farmers. Because the price received is not commensurate with the production costs incurred. In addition, the demand for red chili decreases when farmers are harvesting.

According to the analysis results, the COVID-19 pandemic has affected the price fluctuations of garlic in traditional markets in Jakarta. At the beginning of 2020, the price of garlic rose sharply. This happened because the primary source of garlic imports in China was under lockdown, resulting in logistical difficulties to import to Indonesia. China is the leading importer of garlic to Indonesia. Ninety percent of the garlic sold in the Indonesian market comes from Chinese imports. The Indonesian government should make a program to develop garlic cultivation in Indonesia to not depend on China because the agricultural area in Indonesia is still quite large and can increase garlic productivity. The government needs to provide superior varieties with high yields to increase garlic production. In addition, the government must also raise the enthusiasm of farmers to return to planting garlic. Farmers are also advised to improve the production system and efficiency of garlic cultivation to be more resilient in facing market dynamics.

There are three implications of this research; the first is that this research is expected to be a reference for other researchers who will research the same topic further. The second implication is a consideration for the government in making policies. The third implication is knowledge for people who want to know the causes of fluctuations in basic food prices before and during the COVID-19 pandemic in Jakarta, Indonesia.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Effect of Good Agricultural Practices on Energy Use in Citrus Farming in Turkey: Case of Mersin Province

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Abstract

The study aimed to determine the energy consumption efficiency of citrus production in enterprises that applied and did not apply Good Agricultural Practices (GAP) in Turkey's Mersin province. Total of 89 citrus producers applied good agricultural practices in Mersin in 2013 and the survey was conducted with all the producers and 26 of these producers produced orange, 28 of these producers produced tangerines, and 35 of these producers produced lemon. In the study, for comparing the enterprises, the same survey was also carried out with the same number of producers who did not apply good agricultural practices. Labor, machinery, diesel, farmyard manure, fertilizers, pesticides, electricity, and water in irrigation were calculated as energy inputs, and citrus production quantities were calculated as outputs. According to research results, fertilizers were determined as the most energy-consuming inputs in citrus production. The energy use efficiency values were 1.83 and 1.53 in orange production, 1.75 and 1.48 in tangerine production, 1.66 and 1.34 in lemon production in the enterprises that applied and did not apply good agricultural practices. Therefore, the energy use efficiency that applied good agricultural practices in enterprises was determined to be higher. Energy productivity results showed that citrus producers who applied good agricultural practices could produce more output than citrus producers who did not apply good agricultural practices. Specific energy results indicated that the citrus enterprises that applied good agricultural practices consumed less energy to produce one kg of the product than those that did not apply good agricultural practices. Non-renewable energy shares were lower in enterprises that used good agricultural practices than in the other group. According to the study's findings, Citrus production enterprises that applied good agricultural practices were more profitable in Mersin province.

1. Introduction

Citrus is a plant genus that includes several high-value fruits such as orange, tangerine, lemon, grapefruit, and sour orange. Citrus is endemic to China and India, and it can be grown virtually everywhere in a temperate environment. In Turkey, citrus farming is executed in the south, southwest, and west regions of Anatolia (Anonymous, 2020). Citrus, includes the C vitamin, has significant benefits for human health, and it is evaluated as jam, marmalade, and fruit juice besides the edible consumption and used as a raw material in the cosmetic sector (Uysal and Polatöz, 2017).

According to USDA data, by 2019/2020 production season, a total of 92 million tons citrus production, 46 million ton of which was orange, 32 million ton of which was tangerine, 8 million ton of which was lemon, and 7 million ton of which was sour orange, were done in the World. In other

words, 50%, 34%, 8%, and 7% of this production belonged to orange, tangerine, lemon, and sour orange, respectively. Thus, Turkey takes seventh place in orange production, third place in tangerine production, fourth place in lemon production, and fifth place in sour orange production (USDA, 2020).

In Turkey, 1 million 334 thousand tons of orange, 1 million 586 thousand tons of tangerine, 1 million 189 thousand tons of lemon, and 238 thousand tons of sour orange production were executed in 2020, and 30.69%, 36.48%, 27.35%, and 5.48% of citrus production belonged to orange, tangerine, lemon, and sour orange, respectively. In Mersin province, 193 thousand tons of orange, 302 thousand tons of tangerine, 702 thousand tons of lemon, and 30 thousand tons of sour orange production were performed in 2020, and 14.47%, 19.07%, 59.04%, and 12.61% of citrus production belonged to orange, tangerine, lemon, and sour orange, respectively (Anonymous, 2021a).

Good agricultural practices (GAP) concept is a production model that keeps the agricultural production under control by not damaging the environment, human and animal health and providing sustainability and food security by the certification of the crops. Good agricultural practices are farming itself, not an alternative agricultural production model. Implementations such as chemical pesticides, fertilizers, etc., are present, but they are applied by not damaging human health and the environment in integrated crop production principles in good agricultural practices (Hasdemir, 2011).

Good agricultural practices started in 2007 in Turkey, and especially after 2013, significant developments were registered in terms of producer number and production area. The number of provinces in which good agricultural practices were executed was 18 in 2007, and this number increased to 61 in 2020. Good agricultural practices were performed with 14 501 producers in 254 755 ha area in Turkey in 2020. Mersin province where the production and the consumption of fruit kinds such as citrus foremost, tomato, pepper, stone fruits (apricot, peach, cherry, plum) come into prominence in good agricultural practices. Good agricultural practices were performed with 870 producers in 14 704 ha areas in Mersin province in 2020 (Anonymous, 2021b).

Energy analysis is a practical approach for grouping the agricultural systems in terms of energy consumption. Although the agriculture sector is not a considerable energy consumer, significant energy consumption is present in rural areas due to soil tillage, planting, weed control, irrigation, fertilizing, harvesting, transport, and drying (Yaldız et al., 1993). Energy consumption increases by the modernization of these processes and the increase of agricultural production. Energy analysis determines how efficient energy is used, sustainable farming, a decrease in fossil fuels, environmental protection, and economic benefit provided by efficient energy usage (Bilgili, 2012).

Various studies on fruit production energy analysis were conducted, such as apricot (Gezer et al., 2003; Gundogmus, 2006), sweet cherry (Demircan et al., 2006), dry apricot (Esengün et al., 2007), cherries (Kizilaslan, 2009), pomegranate (Akcaoz et al., 2009), kiwifruit (Mohammadi et al., 2010), banana (Akcaoz, 2011), lemon (Bilgili, 2012), peach (Goktolga et al., 2006; Royan et al., 2012), pear (Liu et al., 2010; Tabatabaie et al., 2013), strawberry (Banaeian et al., 2011; Loghmanpor et al., 2013), grape (Qasemi Kordkheili and Rahbar, 2015), orange (Mohammadshirazi et al., 2015), almond (Beigi et al., 2016), organic grape (Baran et al., 2017a), organic mulberry (Gokdogan et al., 2017b), organic strawberry (Baran et al., 2017b), walnut (Gundogmus, 2013; Baran et al., 2017c), plum (Baran et al., 2017d), peach and cherry (Aydın and Aktürk, 2018), apple (Ekinci et al., 2005; Sami et al., 2011; Strapatsa et al., 2006; Dilay et al., 2010; Rafiee et al., 2010; Yilmaz et al., 2010; Fadavi et al., 2011; Akdemir et al., 2012; Celen et al., 2017; Gokdogan and Baran, 2017a; Aydın et al., 2019), nectarine (Qasemi Kordkheili et al. 2015, Oğuz et al., 2019a), organic wolfberry (Oğuz et al., 2019b), citrus (Ozkan et al., 2004; Namdari et al., 2011; Loghmanpor et al., 2013; Yılmaz and Aydın, 2020), organic almond (Baran et al., 2020),tangerine (Mohammadshirazi et al., 2012; Karabat and Aydın, 2018; Bilgili, 2021).

In this study, the inputs used in orange, tangerine, and lemon production in the enterprises were determined, and the energy equivalents of these inputs were calculated in enterprises that applied and did not apply good agricultural practices in Mersin province. In addition, comparative energy analysis was done in orange, tangerine, and lemon production in the groups, and the efficiency degrees of the inputs were determined.

2. Materials and Methods

The primary data for the study were collected from the citrus producers who applied and did not apply good agricultural practices in the Mersin province. Besides, the previous studies related to the subject and the statistical indicators composed the secondary of the research. Total of 89 citrus producers applied good agricultural practices in Mersin in 2013 and the survey was conducted with all the producers and 26 of these producers produced orange, 28 of these producers produced tangerines, and 35 of these producers produced lemon. The same survey was conducted in the research with the same number of producers who did not apply good agricultural practices to compare the enterprises. The energy inputs of orange, tangerine, and lemon production were labor, machinery, electricity, diesel fuel, irrigation water, farmyard manure, chemicals, and fertilizers, while orange, tangerine, and lemon fruits were production values outputs. The input and output quantities per hectare were computed and multiplied by the

energy equivalent coefficients (Table 1). It was utilized from the previous studies in order to determine the energy equivalent coefficients. Megajoule (MJ) was used to express the energy equivalents of the inputs and outputs. The total input equivalent was computed by calculating the energy equivalents of all inputs in MJ. For determining the energy consumption in orange, tangerine, and lemon production, the following formulas were used: energy use efficiency, specific energy, energy productivity, and net energy coefficients (Mandal et al., 2002). Specific energy represents the quantity per product quantity, whereas energy productivity expresses the quantity per product quantity.

$$Energy\ Use\ Efficiency = \frac{Energy\ Output}{Energy\ Input}$$

$$Energy\ Productivity = \frac{Yield}{Energy\ Input}$$

$$Specific\ Energy = \frac{Energy\ Input}{Yield}$$

$$Net\ Energy = Energy\ Output - Energy\ Input$$

$(Energy: MJ\ ha^{-1}, Yield: kg\ ha^{-1})$

The energy inputs were analyzed in terms of direct, indirect, renewable, and non-renewable energy sources. Labor, diesel, irrigation water, and electricity are examples of direct energy, whereas chemical fertilizers, farmyard manure, pesticides, and machinery are examples of indirect energy. Labor, farmyard manure, and irrigation water are examples of renewable energy, whereas diesel, chemical fertilizers, pesticides, machinery, and electricity are examples of non-renewable energy.

3. Results and Discussion

The quantities of the inputs used in orange production and their energy equivalences are given in Table 2. Besides, Table 2 shows the quantity of the output of orange production and the energy equivalent of orange production. As is seen from

Table 1. Energy equivalents of inputs and outputs in fruit production.

Inputs	Energy equivalent (MJ unit^{-1})	References
Labor (h)	1.96	Singh, 2002
Machinery (h)	64.80	Kizilaslan, 2009; Singh, 2002
Diesel fuel (l)	56.31	Singh, 2002
Farmyard manure (kg)	0.30	Singh, 2002
Fertilizer (kg)		
Nitrogen	60.60	Singh, 2002
Phosphorus	11.15	Singh, 2002
Potassium	6.70	Singh, 2002
Sulfate	1.12	Rafiee et al., 2010
Chemicals (kg)		
Insecticides	101.20	Rafiee et al., 2010
Fungicides	216.00	Rafiee et al., 2010
Herbicides	238.00	Rafiee et al., 2010
Electricity (kWh)	3.60	Ozkan et al., 2004
Irrigation water (m^3)	0.63	Yaldiz et al., 1993
Output		
Fruit (kg)	2.40	Ozkan et al., 2004

Table 2. Quantities of inputs and outputs and total energy equivalents of orange production.

Inputs	GAP orange			Orange		
	Quantity per unit area (ha)	Energy equivalent (MJ)	% of total energy input	Quantity per unit area (ha)	Energy equivalent (MJ)	% of total energy input
Labor (h)	699.80	1371.61	2.64	702.50	1376.90	2.23
Machinery (h)	65.00	4212.00	8.11	63.00	4082.40	6.61
Diesel (l)	102.50	5771.78	11.11	98.50	5546.54	8.99
Farmyard manure (kg)	3200.00	960.00	1.85	3400.00	1020.00	1.65
Fertilizers (kg)						
Nitrogen	336.50	20391.90	39.25	395.50	26158.37	42.38
Phosphorus	380.00	4237.00	8.16	471.00	5859.24	9.49
Potassium	305.00	2043.50	3.93	358.00	3991.70	6.47
Sulfate	156.00	174.72	0.34	168.00	188.16	0.30
Pesticides (kg)						
Insecticides	10.40	1052.48	2.03	11.20	1133.44	1.84
Fungicides	12.10	2613.60	5.03	13.50	2916.20	4.72
Herbicides	13.20	3141.60	6.05	13.80	3284.40	5.32
Electricity (kWh)	955.00	3438.00	6.62	985.00	3546.00	5.74
Irrigation water (m^3)	4035.00	2542.05	4.89	4160.00	2620.80	4.25
Total		51950.23	100.00		61723.95	100.00
Output (Yield)	39520.00	98848.00		39270.00	94248.00	

Table 2, 336.50 and 395.50 kg nitrogen, 380 and 471 kg phosphorus, 305 and 358 kg potassium, 156 and 168 kg sulfate, 3200 and 3400 kg of farmyard manure were used as fertilizers, 102.50 and 98.50 l diesel fuel, 4035 and 4160 m^3 irrigation water, 10.40 and 11.20 kg insecticides, 12.10 and 13.50 kg fungicides, 13.20 and 13.80 kg herbicides, 699.80 and 702.50 h labor, 65 and 63 h machinery, 955 and 985 kWh electrical energy per hectare was used for the orange production that applied and did not apply good agricultural practices, respectively. As a result, the average orange outputs were 39520 and 39 270 ha^{-1}, respectively, in the analyzed enterprises. The total energy consumed during orange production was 51950.23 and 61723.95 MJ ha^{-1}, respectively, and the energy equivalents of the outputs were 98848 and 94248 MJ ha^{-1} in the enterprises that applied and did not apply good agricultural practices (Table 2).

The findings indicated that in orange production, the share of energy consumed consists of 39.25% and 42.38% nitrogen, 11.11% and 8.99% diesel fuel, 8.16% and 9.49% phosphate and 9.29% phosphorus, 8.11% and 6.61% machinery, 6.62% and 5.74% electricity in enterprises that applied and did not apply good agricultural practices, respectively.

The result also showed that proportions of the other energy-consuming inputs for orange production in the enterprises that applied and did not apply good agricultural practices were 3.93% and 6.47% potassium, 4.89% and 4.25% irrigation water, 6.05% and 5.32% herbicides, 5.03% and 4.72% fungicides, 2.03% and 1.84% insecticides, 2.64% and 2.23% labor, 1.85%, and 1.65% farmyard manure, 0.34%, and 0.30% sulfate, respectively.

The most energy-consuming inputs in orange production were fertilizers. After fertilizers and pesticides, diesel, machinery, irrigation water, and electricity were the most energy-consuming inputs. The lowest energy-consuming inputs were determined as labor and farmyard manure. Ozkan et al. (2004) determined that the energy input of chemical fertilizer (49.68%), primarily nitrogen, had the highest percentage of overall energy inputs in citrus (orange, tangerine, and lemon) production followed by diesel (30.79%). In the study carried out by Namdari et al. (2011), diesel was the highest energy input, followed by fertilizers and water for irrigation. Loghmanpour et al. (2013b) stated that fertilizers used the most energy and were the most crucial energy inputs required in citrus-producing fields, followed by pesticides. Mohammadshirazi et al. (2015) determined that chemical fertilizers utilized the most energy (26.9%), followed by chemicals (26.1%).

Table 3 shows the quantities of the inputs required in tangerine production, their energy equivalences, the quantity of tangerine production, and the energy equivalent of the output. According to the results illustrated in Table 3, 347.50 and 360.50 kg nitrogen, 395 and 463.50 kg phosphorus, 343.00 and 327.50 kg potassium, 144 and 144 kg sulfate, 3200 and 3000 kg of farmyard manure, 102.50 and 95.00 l diesel fuel, 4360 and 4580 m^3 irrigation water, 9.30 and 11.20 kg insecticides, 12.80 and 12.20 kg fungicides, 13.50 and 13.00 kg herbicides, 726.80 and 689.00 h labor, 65.00 and 60.50 h machinery, 1085 and 1180 kWh electrical energy per hectare was used for the tangerine production that applied and did not apply good agricultural practices, respectively. As a result, tangerine outputs were 39270 and 36350 ha^{-1} on average, respectively. The total energy used during tangerine productions was 53862.19 and 58938.65 MJ ha^{-1}, and the energy equivalents of the outputs were 94248 and 87240 MJ ha^{-1}, respectively, in the enterprises that applied and did not apply good agricultural practices.

Table 3. Quantities of inputs and outputs and total energy equivalents of tangerine production.

Inputs	GAP tangerine			Tangerine		
	Quantity per unit area (ha)	Energy equivalent (MJ)	% of total energy input	Quantity per unit area (ha)	Energy equivalent (MJ)	% of total energy input
Labor (h)	726.80	1424.53	2.64	689.00	1350.44	2.29
Machinery (h)	65.00	4212.00	7.82	60.50	3920.40	6.65
Diesel (l)	102.50	5771.78	10.72	95.00	5349.45	9.08
Farmyard manure (kg)	3200.00	960.00	1.78	3000.00	900.00	1.53
Fertilizers (kg)						
Nitrogen	347.50	21058.50	39.10	360.50	23843.47	40.45
Phosphorus	395.00	4404.25	8.18	463.50	5765.94	9.78
Potassium	343.00	2298.10	4.27	327.50	3651.63	6.20
Sulfate	144.00	161.28	0.30	144.00	161.28	0.27
Pesticides (kg)						
Insecticides	9.30	941.16	1.75	11.20	1133.44	1.92
Fungicides	12.80	2764.80	5.13	12.20	2635.20	4.47
Herbicides	13.50	3213.00	5.97	13.00	3094.00	5.25
Electricity (kWh)	1085.00	3906.00	7.25	1180.00	4248.00	7.21
Irrigation water (m^3)	4360.00	2746.80	5.10	4580.00	2885.40	4.90
Total		53862.19	100.00		58938.65	100.00
Output (Yield)	39270.00	94248.00		36350.00	87240.00	

According to the results, the proportions of energy consumption in tangerine production consisted of 39.10% and 40.45% nitrogen, 10.72 and 9.08% diesel fuel, 8.18% and 9.78% of phosphorus, 7.82% and 6.65% of machinery, 7.25% and 7.21% of electricity in the enterprises that applied and did not apply good agricultural practices respectively. Besides, the other energy-consuming inputs for tangerine production was 4.27% and 6.20% potassium, 5.10% and 4.90% irrigation water, 5.97% and 5.25% herbicides, 5.13% and 4.47% fungicides, 1.75% and 1.92% insecticides, 2.64% and 2.29% labor, 1.78% and 1.53% farmyard manure, 0.30% and 0.27% sulfate, respectively.

In enterprises that applied good agricultural practices, total fertilizers consumed the most energy, followed by pesticides, diesel fuel, machinery, electricity, irrigation water, labor, and farmyard manure. Fertilizers consumed the most energy, followed by pesticides, fuel, electricity, machinery, labor, and farmyard manure in the other group. Namdari et al. (2011) determined that the highest energy-consuming inputs were diesel fuel, chemical fertilizer, and water for irrigation with 24, 23, and 23% shares, respectively, in tangerine production. Mohammadshirazi et al. (2012) determined that fertilizers had the highest energy consumption in tangerine production. In the studies carried out by Karabat and Aydın (2018) and Yılmaz and Aydın (2020), fertilizers, pesticides, and diesel were determined to be the first three highest energy-consuming inputs in the enterprises. Bilgili (2021) determined that fertilizers were the highest energy-consuming inputs in tangerine production.

Table 4 shows the inputs required in lemon production (physical quantity per hectare), yield per hectare (output), and energy equivalents. The results showed that about 728.50 and 734.60 h labor, 59.50 and 61.50 h machinery, 88.00 and 95.50 l diesel, 3000 and 3500 kg farmyard manure, 374.00 and 433.50 kg nitrogen, 456.00 and 516.50 kg phosphorus, 320 and 396 kg potassium, 168 and 180 kg sulfate, 9.50 and 11.20 kg insecticides, 13.60 and 16.50 kg fungicides, 11.80 and 13.80 kg herbicides, 1150 and 1235 kWh electricity and 4050 and 4360 m^3 irrigation water were used per hectare for lemon production that applied and did not apply good agricultural practices. The total energy used in the enterprises were calculated as 54618.60 and 66741.21 MJ ha^{-1}, most of which was related to fertilizers (55.08% and 59.51%), followed by pesticides (12.28% and 11.96%), diesel (9.07% and 8.06%), electricity (7.58% and 6.66%), machinery (7.06% and 5.97%), irrigation water (4.67% and 4.12%), labor (2.61% and 2.16%) and farmyard manure (1.65% and 1.57%) in the enterprises that applied and did not apply good agricultural practices, respectively. Lemon yields were determined to be 37690 and 37290 kg ha^{-1} on average. As a result, 90456 and 89496 MJ ha^{-1} were calculated as total energy output per hectare, respectively.

Bilgili (2012) reported that fertilizers were the highest energy inputs in lemon production, followed by fuel, pesticides, irrigation water, labor, and machinery. In contrast, the study by (Yılmaz and Aydın, 2020) found that fertilizers, chemicals, and diesel fuel were the most energy-consuming inputs in lemon production, respectively.

Table 5 shows the energy parameters in citrus production. The energy use efficiency (energy ratio) values were 1.83 and 1.53 in orange production, 1.75 and 1.48 in tangerine production, 1.66 and 1.34 in lemon production in the enterprises that applied and did not apply good agricultural practices, respectively. This revealed that energy usage in citrus production was efficient regardless of product type. In other words, energy production was more remarkable than energy utilization.

Table 4. Quantities of inputs and outputs and total energy equivalents of lemon production.

Inputs	GAP lemon			Lemon		
	Quantity per unit area (ha)	Energy equivalent (MJ)	% of total energy input	Quantity per unit area (ha)	Energy equivalent (MJ)	% of total energy input
Labor (h)	728.50	1427.86	2.61	734.60	1439.82	2.16
Machinery (h)	59.50	3855.60	7.06	61.50	3985.20	5.97
Diesel (l)	88.00	4955.28	9.07	95.50	5377.61	8.06
Farmyard manure (kg)	3000.00	900.00	1.65	3500.00	1050.00	1.57
Fertilizers (kg)						
Nitrogen	374.00	22664.40	41.50	433.50	28671.69	42.96
Phosphorus	456.00	5084.40	9.31	516.50	6425.26	9.63
Potassium	320.00	2144.00	3.93	396.00	4415.40	6.62
Sulfate	168.00	188.16	0.34	180.00	201.60	0.30
Pesticides (kg)						
Insecticides	9.50	961.40	1.76	11.20	1133.44	1.70
Fungicides	13.60	2937.60	5.38	16.50	3564.00	5.34
Herbicides	11.80	2808.40	5.14	13.80	3284.40	4.92
Electricity (kWh)	1150.00	4140.00	7.58	1235.00	4446.00	6.66
Irrigation water (m^3)	4050.00	2551.50	4.67	4360.00	2746.80	4.12
Total		54618.60	100.00		66741.21	100.00
Output (Yield)	37690.00	90456.00		37290.00	89496.00	

Table 5. Energy parameters in orange, tangerine, and lemon production.

Calculations	GAP orange	Orange	GAP tangerine	Tangerine	GAP lemon	Lemon
Total energy input (MJ ha^{-1})	51950.23	61723.95	53862.19	58938.65	54618.60	66741.21
Total energy output (MJ ha^{-1})	94848.00	94248.00	94248.00	87240.00	90456.00	89496.00
Energy use efficiency	1.83	1.53	1.75	1.48	1.66	1.34
Energy productivity (kg MJ^{-1})	0.76	0.64	0.73	0.62	0.69	0.56
Specific energy (MJ kg^{-1})	1.31	1.57	1.37	1.62	1.45	1.79
Net energy (MJ ha^{-1})	42897.77	32524.05	40385.81	28301.36	35837.40	22754.79

Nonetheless, the energy usage efficiency in citrus production was better when good agricultural practices were applied.

Previous researches on citrus production revealed energy usage efficiency values of 1.25, 1.17, and 1.06 in orange, tangerine, and lemon, respectively (Ozkan et al., 2004), 1.25 and 1.17 in orange and tangerine (Namdari et al., 2011), 0.87 in tangerine (Mohammadshirazi et al., 2012), 1.716 in all citrus (Loghmanpour et al., 2013b), 1.02 in lemon (Bilgili, 2012), 0.67 in orange (Mohammadshirazi et al., 2015), 2.24 and 2.04 in tangerine (Karabat and Aydın, 2018), 2.03 and 1.88 in tangerine, 1.82 and 1.58 in lemon (Yılmaz and Aydın, 2020), and 1.56 in tangerine production (Bilgili, 2021).

Energy consumption efficiencies in orange, tangerine, and lemon production were found to be higher than one. This was similar to earlier citrus production research findings, such as Ozkan et al. (2004), Namdari et al. (2011), Loghmanpour et al. (2013b), Bilgili (2012), Karabat and Aydn (2018), Yılmaz and Aydn (2020), Bilgili (2012) and Bilgili (2021). Furthermore, the energy usage efficiency of citrus production applied good agricultural practices was more remarkable in this study than citrus production did not apply good agricultural practices. The findings are similar to the studies conducted by Karabat and Aydın (2018) and Yılmaz and Aydın (2020).

Energy productivity is the term used to estimate the product yield per unit of energy consumption. Average energy productivity values were 0.76 and 0.64 kg MJ^{-1} in GAP orange and orange production, 0.73 and 0.62 kg MJ^{-1} in GAP tangerine and tangerine production, 0.69 and 0.56 kg MJ^{-1} in GAP lemon and lemon production, respectively. This means that, for example, in orange production that applied good agricultural practices, 0.76 kg output was obtained for every 1 MJ of energy consumed. When the production types were compared, it was discovered that citrus orchards that applied good agricultural practices could generate greater output than citrus orchards that did not apply good agricultural practices.

Specific energy was calculated as 1.31 and 1.57 MJ kg^{-1} in orange production, 1.37 and 1.62 MJ kg^{-1} in tangerine production, and 1.45 and 1.79 MJ kg^{-1} in lemon production, respectively in enterprises that applied and did not apply good agricultural practices. This means that, for example, in orange production, applied good agricultural practices. For producing one kg of orange, 1.31 MJ of energy was consumed. When the two production types were compared, enterprises that applied good agricultural practices used less energy to produce one kilogram of the product than those that did not apply good agricultural practices.

Net energy values for orange production were 42897.77 and 32524.05 MJ ha^{-1}, tangerine production was 40385.81 and 28301.36 MJ ha^{-1}, and lemon production was 35837.40 22754.79 MJ ha^{-1} in enterprises that applied good agricultural practices. According to calculations of energy use efficiency, citrus production was more profitable for companies that applied good agricultural practices.

Table 6 shows the distribution of input energy in citrus production by direct, indirect, renewable, and non-renewable energy sources. The majority of the energy input came from non-renewable and indirect sources. As shown in the table, the total energy input in orange production can be classified as direct (25.26% and 21.21%), indirect (74.74% and 78.79%), renewable (9.38% and 8.13%), and non-renewable (90.62% and 91.87%) applied and did not apply good agricultural practices.

On average, the proportions of direct and indirect energy in enterprises in tangerine production that applied good agricultural practices was 25.71% and 74.29%, while direct and indirect energy was 23.47% and 76.53% in tangerine production did not apply good agricultural practices. Also, renewable and non-renewable energy contributed to 9.53% and 90.47% of the total energy input in GAP tangerine production, whereas renewable and non-renewable energy contributed 8.71% and 91.29% tangerine production did not apply good agricultural practices.

Table 6 also demonstrated that the proportions of direct energy is lower (23.94% and 20.99%) than indirect energy (76.06% and 79.01%) of lemon producers who apply and do not apply good agricultural practices. Also, non-renewable and renewable energies contributed to 91.07% and 8.93% of the total energy input in GAP lemon production and 92.15% and 7.85% in lemon production.

Ozkan et al. (2004), Namdari et al. (2011), Bilgili (2012), Mohammadshirazi et al. (2012), Loghmanpour et al. (2013b), Mohammadshirazi et al. (2015), Karabat and Aydın (2018), Yılmaz and Aydın (2020) and Bilgili (2021) determined that the ratio of non-renewable energy was more significant than the ratio of renewable energy in citrus production.

The high non-renewable energy ratio in overall energy inputs has a detrimental impact on agricultural productivity and the environment.

Table 6. Energy input forms of orange, tangerine, and lemon production.

Energy form	GAP orange	Orange	GAP tangerine	Tangerine	GAP lemon	Lemon
Direct energy [a]	13123.43 (25.26%)	13090.24 (21.21%)	13849.10 (25.71%)	13833.29 (23.47%)	13074.64 (23.94%)	14010.22 (20.99%)
Indirect energy [b]	38826.80 (74.74%)	48633.71 (78.79%)	40013.09 (74.29%)	45105.36 (76.53%)	41543.96 (76.06%)	52730.99 (79.01%)
Renewable energy [c]	4873.66 (9.38%)	5017.70 (8.13%)	5131.33 (9.53%)	5135.84 (8.71%)	4879.36 (8.93%)	5236.62 (7.85%)
Non-renewable energy [d]	47076.58 (90.62%)	56706.25 (91.87%)	48730.87 (90.47%)	53802.81 (91.29%)	49739.24 (91.07%)	61504.60 (92.15%)
Total energy input	51950.23 (100.00)	61723.95 (100.00%)	53862.19 (100.00%)	58938.65 (100.00%)	54618.60 (100.00%)	66741.21 (100.00%)

[a] Includes labor, diesel, electricity, and irrigation water.
[b] Includes fertilizers, chemicals, farmyard manure, and machinery.
[c] Includes labor, farmyard manure, and irrigation water.
[d] Includes diesel, chemicals, fertilizers, machinery, and electricity.

However, the non-renewable energy ratio in the enterprises that applied good agricultural practices was lower than the enterprises in the other group. This result was similar to the studies carried out by Karabat and Aydın (2018) in tangerine production and Yılmaz and Aydın (2020) in citrus production.

4. Conclusion

Efficient and productive use of the energy sources is significant for all the countries in terms of economic development besides the supply of essential requirements. Evaluated in terms of agricultural production, citrus compose approximately 4% of the total fruit production areas in Turkey. For this reason, energy use is very important in terms of citrus products.

According to the results, the energy use efficiency in citrus production was higher in the enterprises that applied good agricultural practices in Mersin province. This result can be stated as the production inputs were used more controlled in the enterprises applied good agricultural practices. Therefore, it is suggested that especially the producers who did not apply good agricultural practices should be trained in input usage.

Renewable energy inputs were mainly diesel and electricity, whereas fertilizers and pesticides dominated nonrenewable inputs. This situation demonstrates that citrus production was significantly dependent on nonrenewable energy input. Inputs remained low with fertilizers, chemicals, diesel fuel, electricity, irrigation water, machinery, labor, and farmyard manure. The use of appropriate fertilizers and pesticides may lower the indirect energy requirements for pest control and manure.

According to the findings, the existing energy consumption pattern in the orchards is dependent on non-renewable energy. In other words, the proportion of renewable energy used in the orchards surveyed was low. Therefore, reducing the total non-renewable energy ratio, especially fertilizer use, would positively affect the sustainability of citrus production and positive environmental effects.

Considering the results of this study, it was determined that the generalization of good agricultural practices was essential. As a result, the subsidy levels offered to producers who apply good agricultural practices should be increased, and purchase guarantees for products produced using good agricultural practices should be offered. Furthermore, producer training in this area should be kept up to date. Regulation changes can be done for continuous producer training and incorporating the agricultural agents into the training. The producers' demands and suggestions should be evaluated as essential technical applications and should be within the regulations and directions of good agricultural practices.

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