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Preharvest Use of γ-Aminobutyric Acid (GABA) as an Innovative Treatment to Enhance Yield and Quality in Lemon Fruit

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Abstract: γ-Aminobutyric acid (GABA) occurs naturally at a low concentration in fruits, but can be increased following several stress events, playing a physiological effect. Lemon trees were preharvest treated with GABA at three concentrations (10, 50, and 100 mM) during two consecutive seasons (2019–2020 and 2020–2021). Fruit growth (diameter) and crop yield (kg tree^{-1} and number of fruits tree^{-1}) and quality traits were evaluated at harvest. Results showed that treatments were effective at increasing lemon size (a 5% higher) and yield, especially for GABA at 100 mM, for the two assayed seasons. Thus, yield was increased between 13 and 18% with respect to the control trees for the two harvest dates. With respect to the quality traits, GABA treatments did not impact any negative effects on the quality attributes, since the total soluble solids (7–8° Brix), total acidity (5–6 g 100 g^{-1}), and fruit firmness (13–14 N mm^{-1}) were similar to the control fruits. Therefore, GABA applied as preharvest treatment could be considered as a potent tool to enhance the yield of lemon fruits.

Keywords: elicitor; fruit growth; quality at harvest; climate change; citrus fruit

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1. Introduction

The five biggest exporters of lemons are Spain, Mexico, South Africa, the Netherlands and Turkey, with Spain being the first exporter worldwide and a value of more than EUR 4 billion. According to the latest statistics (2020/2021), Spain will produce 1,035,000 t, broken down into 820,000 t of 'Fino' lemons and 215,000 t of 'Verna' lemons [1].

The lemon market is subjected to EU regulations, with safety issues derived from using different contaminants, and thus not exceeding the MRL (maximum residue levels) and avoiding to reach the consumers [2]. In this sense, although consumers demand organic foods, organic lemon production is very small compared to conventional lemons. However, the total area for producing organic lemons has grown in Spain, reaching 8300 hectares in 2020, which represents a 14% of the total lemon production in Spain, with Murcia, Andalucía, and Valencia the main producing regions.

In lemon, as in other citrus fruits, the growth and development pattern follow a single sigmoid curve (either measured by weight, length, volume, or diameter), which can be divided into three stages [3]. In Stage 1, anthesis (increased cell number) and fruit drop occur, with little or moderate growth. Stage 2 corresponds to from fruit drop to the initial color changes, with a rapid fruitlet growth (cell expansion) and reaching the final size. Finally, at Stage 3, fruit maturation takes place, at which acidity decreases and sweetness increases and the color changes (from green to yellow) [4]. During lemon growth and development, the maximum accumulation of sugars takes place when the fruit size is around 50% of the final volume and then declines as maturation advances. In most cultivars, the highest accumulation of sugars followed the acidity losses. Quality traits in lemon include both external (size, shape, skin thickness, visual appearance, and color), and internal quality (percentage of juice, number of seeds, sugars, acidity, flavor, taste, and bioactive compounds with antioxidant activity), which contribute to the consumer acceptability [5].

γ-Aminobutyric acid (GABA) is a non-proteinaceous amino acid of four carbons that occurs naturally in both plants and animals. Other isomers such as α-aminobutyric acid (AABA), also known as homoalanine, and β-aminobutyric acid (BABA) have also been reported to play a physiological role in animals (AABA), while BABA is a naturally-occurring moiety in plants [6]. GABA is found naturally in small concentrations in many plant sources including fruits, vegetables, and cereals. In plant cells, GABA accumulates under several conditions of abiotic stress, and plays a physiological role in redox balance, osmoprotection, osmotic adjustment, and antioxidant functions, among others [7].

In humans, it is well-known that GABA has multiple health-promoting properties including that GABA plays a role as an inhibitory neurotransmitter of the neuronal cortex acting on the central nervous system, but is also considered as a bioactive compound in foods with different roles such as anti-inflammatory, anti-diabetic, anti-hypertensive, and anti-cancer [8].

The impact of climate change and other environmental factors such as biotic and abiotic stress affect crop production and quality [9]. Citrus production is affected by several environmental conditions such as low and high temperatures, drought, and flooding, among others, that have negatively impacts and are considered as a big challenge for humans [10]. Accordingly, GABA exogenous application to the crop has demonstrated a significant enhancement of the endogenous content of GABA, which in turn alleviates the consequences of the stress [11]. On the other hand, there is some evidence that the accumulation of GABA occurred during the developmental stages of plants including fruit growth and ripening.

Tomato is used as a model since it accumulates GABA at higher rates during growth and ripening. GABA levels increase from flowering to the mature green stage, and then rapidly diminishes during the ripening [11]. Accordingly, GABA was found at elevated concentration near the breaker stage and rapidly catabolized, reaching low levels [12]. In higher plants, GABA is synthesized from glutamate by the enzyme glutamate decarboxylase, and metabolized through the GABA shunt pathway in two consecutive steps, first the oxidation to α-ketoglutarate, and then to succinate and enter the tricarboxylic acid cycle (TCA). The GABA shunt is involved in a wide range of physiological responses through the mitigation of reactive oxygen species (ROS) and plays a key role either as metabolites or endogenous signaling molecules in several regulatory mechanisms under stress conditions [13].

As a preharvest treatment, the role of GABA on growth and development has been reported in several fruits and vegetables, which has recently been reviewed [14], the most studied fruit being tomato. In addition, the preharvest application of GABA to apple trees led to the inhibition of fruit soft scald development depending on spray timing, but with benefits during postharvest storage [15].

On the other hand, the application of elicitors such as methyl jasmonate (MeJA) or salicylic acid (SA) induced the accumulation of endogenous GABA, and in turn, an increase in the total yield per plant was found in tomato with higher fruit quality attributes such as firmness, total soluble solids, and titratable acidity [16]. In an early report, the first quantitative content of GABA in lemon juice was lower (7 mg 100 mL^{-1}) than those reported for orange juices of several cultivars (18–32 mg 100 mL^{-1}) such as 'Valencia' and 'Washington Navel' [17].

As far as we know, there is no literature on the role of GABA preharvest application on citrus fruits. In this sense, the present study aimed to explore the impact of the foliar application of GABA in two consecutive years (2019 and 2020) in lemon trees on fruit growth and yield (kg tree^{-1} and number of fruits) and to evaluate the role of GABA on the lemon quality attributes at harvest time.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiments were carried on a commercial orchard located in Orihuela (Alicante, Spain, 38°7′49.09″ N, 0°59′54.38″ W) during two consecutive years (2019–2020 and 2020–2021) on lemon trees (*Citrus limon* (L.) Burm. f) of the 'Fino-95' cultivar grafted on *C. macrophylla* rootstock and 15 years old. For both years, three blocks of three trees were selected at random for each treatment: GABA, purchased from Sigma (Sigma-Aldrich, Madrid, Spain) at 10, 50-, and 100-mM concentrations and the control (distilled water).

Geomorphologically, it is characterized by a clear predominance of a semi-arid morphogenetic system, highlighting accumulation processes with glacis-type forms and terrain with a slight slope. This region is characterized by being of the Mediterranean type transitioning to the desert climate, hot and dry, with little rainfall. The monthly temperature and rainfall for the whole experimental years is shown in Figure 1, at which the COL event is marked with an arrow, and the average temperature of 19 °C and rainfall of 230 mm.

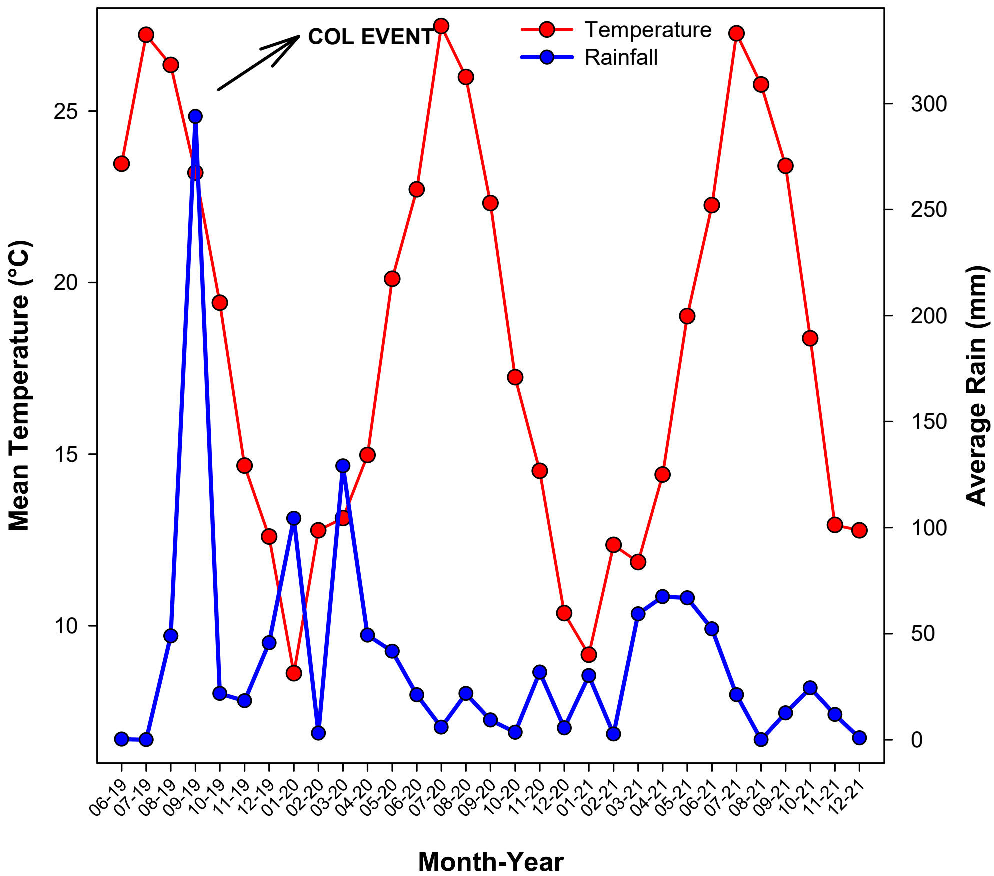

Figure 1. Mean temperature (°C) and rainfall (mm) along the experimental period (2019 and 2020).

Treatments were performed by foliar spray application with freshly prepared solution (5-L per tree) containing 0.5% Tween 20 as the surfactant and the phenological stage (BBCH-scale) was stage 71. The sprays were carried out with a 15-L backpack sprayer until runoff. Each treatment was repeated three times, starting after the typical fruitlet drop, at the onset of color change, and four days before harvest.

2.2. Fruit Growth and Crop Yield

For both years (2019 and 2020), before the first application, five fruits from each tree were marked at random. Fruit growth was followed by measuring the diameter with a digital caliper every 2 weeks, and the results were expressed in mm ± SE. Production was evaluated based on the total yield (kg tree^{-1}) and recording the number of fruits per tree. Two harvest dates were performed (November and February), which are the normal practices for the 'Fino-95' cultivar at which three categories were chosen: green, yellow, and wasted (non-commercial). From each tree and treatment, three lots of 15 fruits were picked and transported to the laboratory for the following analysis.

2.3. Fruit Quality Traits

All the parameters were measured individually in the 15 fruits of each replicate and the results expressed as the mean ± SE. Total soluble solids (TSS), total acidity (TA), firmness, and color were analyzed according to a previous report [18]. In brief, TSS was determined in the juice using with a digital refractometer at 20 °C (model Atago PR-101, Atago Co. Ltd., Tokyo, Japan) with the results being expressed as g 100 g^{-1}. After recording the pH of the juice, the TA was measured by potentiometric titration with 0.1 N NaOH up to reaching pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O, and the results were expressed as g citric acid content equivalent per 100 g^{-1}. Fruit firmness was determined as the force–deformation ratio (N mm^{-1}) by using a TA-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK). The external color was determined by the use of a Minolta colorimeter (CRC-400, Konica Minolta Co., Tokyo, Japan), recording L, a, and b coordinates, and color was expressed as the citrus color index (CCI, $1000 \times a/L \times b$).

2.4. Statistical Analysis

A factorial design with GABA treatments (0, 10, 50, and 100 mM) with three triplicates of three trees per replicate was performed for both years (2019 and 2020). An analysis of variance (ANOVA) was performed by using the SPSS software statistic version 21.0 (IBM$^{\Theta}$ SPSS$^{\Theta}$, USA). and means were compared by Tukey's test to find significant differences among treatments at $p < 0.05$.

3. Results

3.1. Fruit Growth and Crop Yield

Fruit growth was evaluated during the two consecutive years of the experiments by measuring the fruit diameter, and the results showed the typical single-sigmoid growth pattern of the citrus fruits (Figure 2). No significant differences were found between the control and GABA-treated trees for the 10- and 50-mM (average 46 and 57 mm for 2019 and 2020, respectively), although the preharvest application of GABA at 100 mM showed fruits with larger size (52 and 59 mm for 2019 and 2020, respectively). In September 2019, aa cold drop occurred, also known as a cut-off low (COL), which created unsettled weather and produced many thunderstorms with torrential rain and flash flooding [19].

With respect to the crop yield, two parameters were evaluated: total yield per tree (Figure 3) and the total fruit number (Figure 4). Two harvest dates is the normal procedure for this lemon cultivar, the first in November and the second in February, and three lemon categories were established: green, yellow, and waste (non-commercial), according to marketing procedures. In 2019 and harvest-1, the yield was similar for the control and 10- or 50-mM GABA (≅30 kg tree^{-1}), although the 100 mM GABA significantly increased ($p < 0.05$) the yield of green lemons (37.5 ± 4 kg tree^{-1}).

With respect to yellow lemons, all GABA treatments showed significantly ($p < 0.05$) higher yield (≅5 kg tree^{-1}) than the controls (3.31 ± 0.4 kg tree^{-1}), while the waste category was very variable (2.5–4.5 kg tree^{-1}), although the GABA at 10 mM doses significantly showed ($p < 0.05$) a lower rate of wasting lemons (Figure 3). During harvest-2, lemons acquired the full color and two categories were established (yellow and waste). In harvest-2, all GABA-treated trees produced a higher ($p < 0.05$) yield (≅63–74 kg tree^{-1}) compared to the controls (47.4 ± 2 kg tree^{-1}), and again, the wasting lemons remained variable (14–19 kg tree^{-1}).

In 2020, the experiment was repeated and the results were very similar, Thus, the 100 mM GABA significantly ($p < 0.05$) exhibited the highest yield (89.1 ± 1 kg tree^{-1}) of green lemons at harvest-1, and 193 ± 21 kg tree^{-1} of yellow lemons at harvest-2. With respect to waste (non-commercial), all GABA treated trees significantly ($p < 0.05$) showed lower wasting lemons (≅21–28 kg tree^{-1}) than the control trees (37.1 ± 2 kg tree^{-1}). It is worthy to highlight that net production was much higher during 2020 than 2019 due to the fatal incidence of the COL event (Figure 3).

In relation to the number of fruits, results showed that GABA at 100 mM was also effective on increasing ($p < 0.05$) the number of green lemons in 2019 for both harvest dates (Figure 4). In 2020, there were not significant differences ($p < 0.05$) in the number of green fruits at harvest-1 (≅500 fruits), with the exception of 50 mM GABA, where the number of fruits was significantly ($p < 0.05$) lower (445 ± 12 fruits tree^{-1}). At havest-2, GABA at 10 or 100 mM showed the highest number of fruits (≅1350 fruits) and was significantly lower ($p < 0.05$) in the control trees (990 ± 22 fruits tree^{-1}). The number of wasting fruits was also very variable, and only for harvest-2 in 2020, all GABA treated trees showed significantly ($p < 0.05$) lower number of wasting fruits (≅155) than the controls (217 ± 11 fruits tree^{-1}).

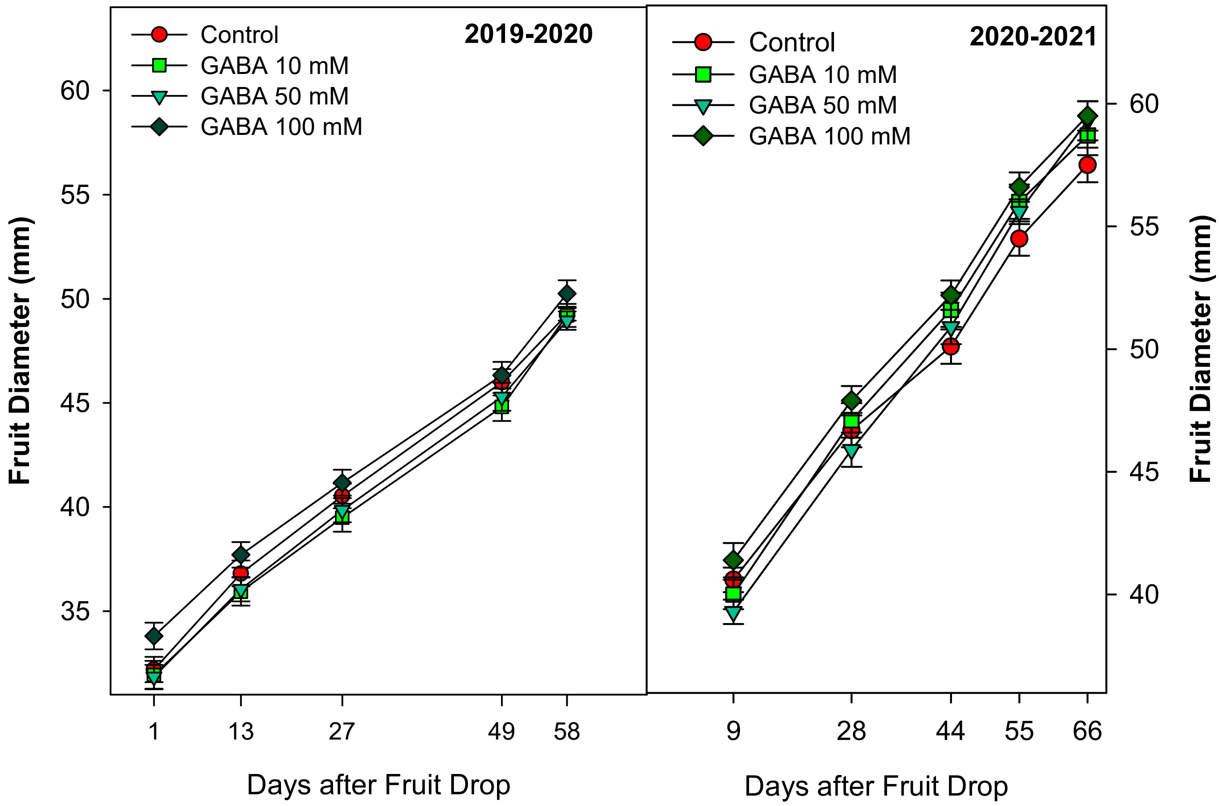

Figure 2. Fruit diameter (mm) in the 'Fino-95' lemon fruit during on-tree fruit development in the control and GABA-treated trees at 10, 50, and 100 mM. Data are the mean ± SE.

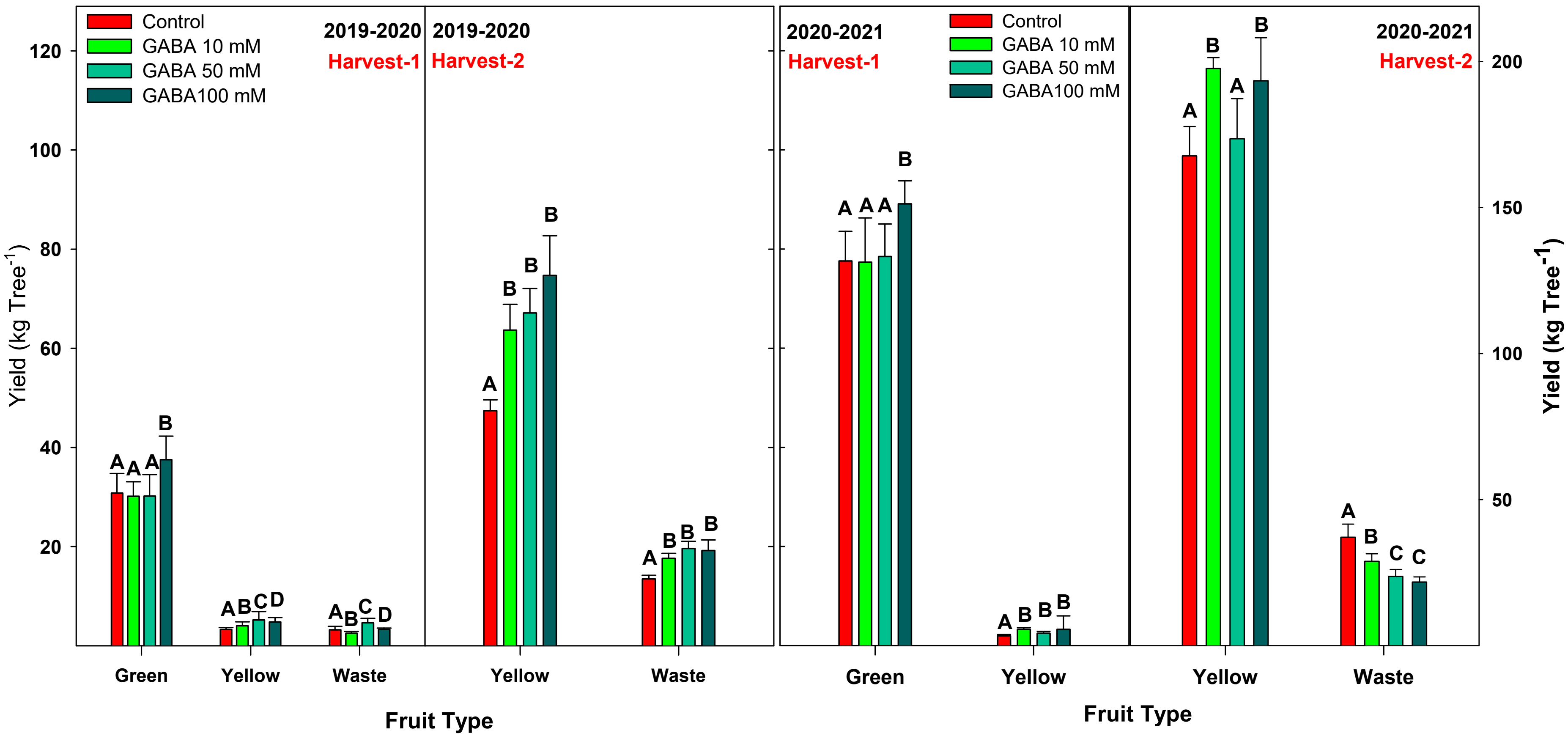

Figure 3. Crop yield (kg tree^{-1}) for the two harvest dates and two seasons as affected by GABA treatment and according to different categories. Data are the mean ± SE. Different letters (A, B, C, and D) on the bars denote significant differences at $p < 0.05$ among treatments for each fruit type.

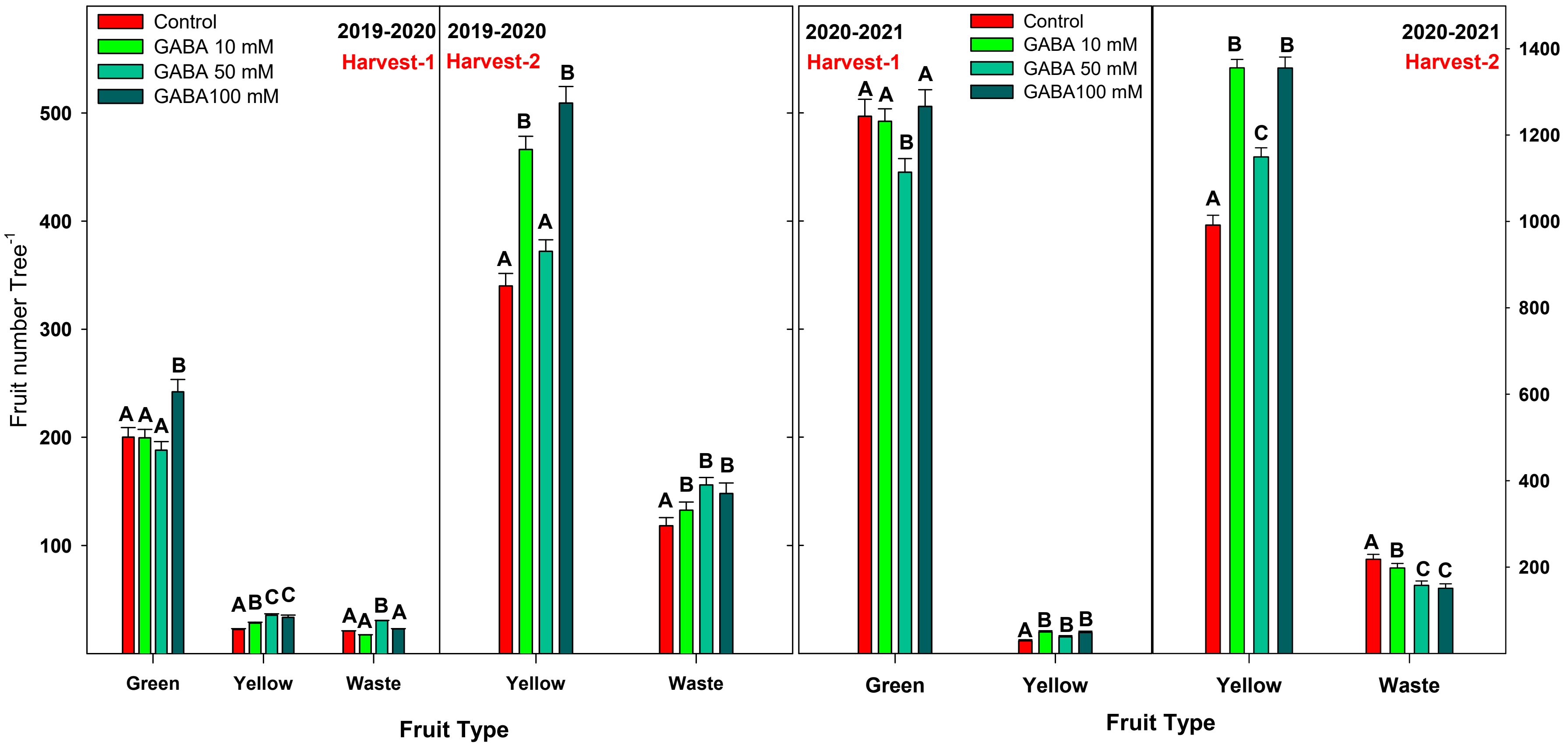

Figure 4. Crop yield (number of fruits tree^{-1}) for the two harvest dates and 2 seasons as affected by GABA treatment and according to different categories. Data are the mean ± SE. Different letters (A, B, and C) on the bars denote significant differences at $p < 0.05$ among treatments for each fruit type.

3.2. Fruit Quality Traits

Lemon fruit quality parameters were evaluated for the two seasons (2019 and 2020) from the picked fruits at both harvest dates. As reported above, at the first harvest date (November), the 'Fino-95' cultivar was picked at the green stage, which had negative values of CCI (Figure 5); the values being slightly lower in the GABA-treated ($\cong -7.5$ and $\cong -5.5$) than in the control lemons ($\cong -6$ and $\cong -5$ for the 2019–2020 and 2020–2021 seasons, respectively. At harvest-2 (February), CCI was $\cong 0.4$ in 2019 for all of the treated and control fruits, while in 2020, GABA at 100 mM significantly ($p < 0.05$) had the highest CCI.

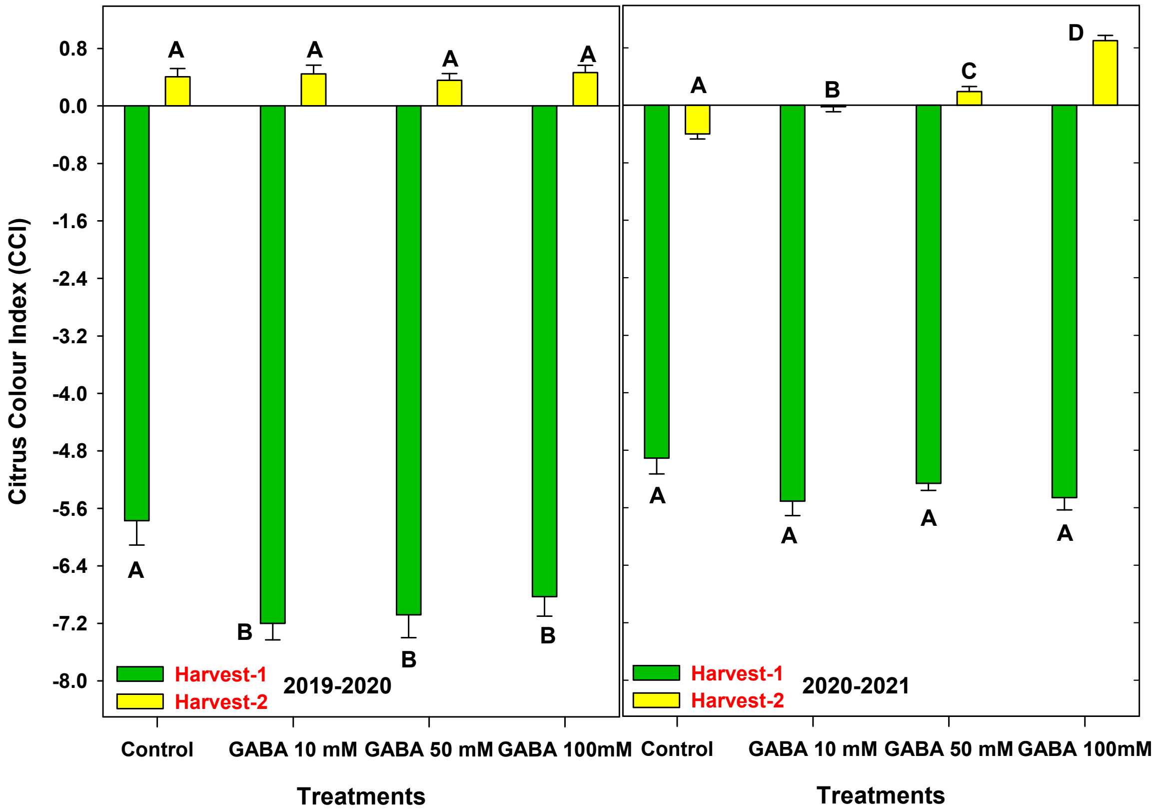

Figure 5. External color (expressed as the citrus color index) of lemons for the two harvest dates and two seasons as affected by GABA treatment. Data are the mean ± SE. Different letters (A, B, C, and D) on the bars denote significant differences at $p < 0.05$ among the treatments.

Other quality parameters were the concentration of total soluble solids (TSS) and total acidity (TA) as well as fruit firmness (Figure 6). No significant ($p < 0.05$) effect on TSS was observed for both seasons and the two harvest dates with the concentration being $\cong$7.7–8 g 100 g^{-1} for all fruits. Regarding the TA, in general, there were no significant differences ($p < 0.05$) between the control and treated lemons, with the exception of GABA at 50 mM. In addition, the TA levels were significantly ($p < 0.05$) higher in those fruit picked at harvest-1 (6.9–7.2 and 5.9–6.4 g 100 g^{-1}) for 2019 and 2020, respectively) compared with those at harvest-2 (5.9–6.4 and 5–5.6 g 100 g^{-1}, for 2019 and 2020, respectively). Similarly, fruit firmness did not show significant differences ($p < 0.05$) for all fruits, the only exception being found in lemons treated with GABA at 50 mM, in which firmness was significant lower ($p < 0.05$). Furthermore, as occurred for TA, the firmness values were significantly ($p < 0.05$) higher in harvest-1 (13.5–16.3 N mm^{-1}) with respect to harvest-2 (13.5–9.6 N mm^{-1}).

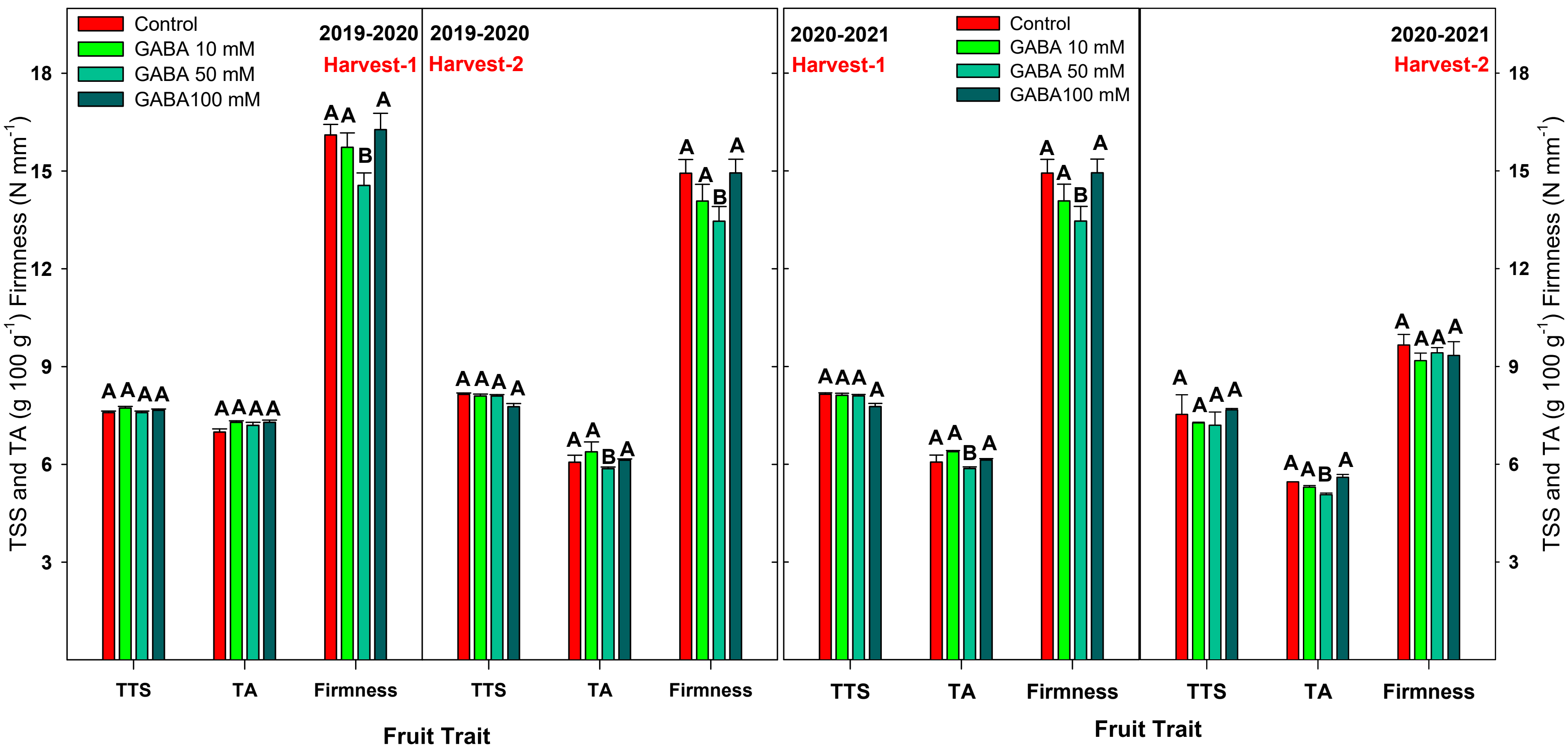

Figure 6. Quality traits of lemons for the two harvest dates and two seasons as affected by GABA treatment. Data are the mean ± SE. Different letters (A and B) on the bars denote significant differences at $p < 0.05$ among treatments for each quality parameter.

4. Discussion

With the aim to increase the productivity of the citrus industry, lemon trees were preharvest treated with GABA at three concentrations: 10, 50, and 100 mM. Overall, the results showed that GABA increased the fruit size and yield, the best concentration being 100 mM. The growth curve of thee 'Fino-95' lemon, which is characterized as having abundant seeds, is represented by a sigmoidal curve [20]. The differences in fruit size between the 2019 and 2020 growing cycles can be attributed to the unexpected cold drop, which provoked a reduction in fruit size. However, the application of GABA could increase the sink capacity of the fruits with a net increase in lemon size, as has been reported for other plant hormones such as auxins and gibberellins [4].

GABA treatments on lemon trees were very effective at enhancing the crop yield, determined by both production (kg tree^{-1}) and the number of fruits tree^{-1} for the two seasons and the two harvest dates, the effect being higher after the application of GABA at 100 mM. It was very noticeable that 2019 rendered a lower production than 2020 due to the incidence of the COL event, which did not occur in 2020. It has been reported that flooding can severely affect the crop yield, resulting in a 20–25% reduction in yield on average [21]. The decline in crop yield has been attributed to a reduction in the photosynthetic rate accompanied by the damage of protective enzymes [22], and then the exogenous application of GABA, especially at 100 mM, could partially increase the lemon yield, since the accumulation of GABA has been described as a response to several abiotic stresses including waterlogging. Moreover, under this stress situation, the effect of GABA at 100 mM on improving fruit production acquired special importance, since during harvest-2, the yield was almost 2-fold compared with the control fruits. A similar behavior was observed for the number of fruits per tree, for which the GABA treatments also showed a positive effect on increasing the number of fruits compared with those obtained in the control lemons, especially for 100 mM GABA. It is worthy to point out that the effects for both crop yield parameters were not dose-dependent, since GABA at 10 mM showed higher proficiency than at 50 mM. There is no literature on the role of GABA in fruit productivity, although some evidence exists in cereals and vegetables. In onion, the application of GABA at 0.5, 1.0, and 2.0 mg L^{-1} increased the bulb yield and other morphological characters, the highest effect being reported for GABA at 1.0 mg L^{-1} [23] in agreement with the results reported herein. In line with this report, foliar application of GABA at 0.5, 1, and 2 mM ameliorated drought stress and improved the yield of snap bean [24].

Citrus fruit is botanically considered as a hesperidium with a specific type of modified berry that is divided internally into segments containing the juice. The rind or peel of lemon is formed by the exocarp or colored flavedo and the colorless or white albedo. The ripening process is defined as the set of external flavor and texture changes that a fruit experiences when it completes its growth. This phase of the development includes several processes such as coloration of the pericarp, an increase in the concentration of sugars, a reduction in acid concentration, loss of firmness, and other physical and chemical changes [5].

Lemon fruits are generally harvested at different maturity stages to fulfil the market requirements. In the case of the 'Fino-95' cultivar, two harvest dates are the normal procedure, one in November and the other in February. The size and the color of the fruit are two of the main characteristics that determine when the fruit should be harvested. During autumn, as the temperature starts to decrease (below 13 °C), the fruit starts to change in color due to chlorophyll breakdown and the occurrence of the peel yellow color, from which the main pigments are carotenoids, while in the second harvest, the fruit is fully colored [25].

Other important parameters in lemon quality are related to the content of total soluble solids (TSS), total acidity (TA), and fruit firmness. From the point of view of the market, TSS and TA are undoubtedly the most important parameters. In mature lemons, sugars account for 80–90% of TSS depending on cultivars, the rest corresponded to TA, the major being citric acid, which is the main organic acid present in citrus fruits [26]. The content of TSS and TA were not affected by the GABA treatments, and the content agrees with previous reports for 'Fino-95' compared with other cultivars such as 'Verna' and 'Fino-49' [27]. Fruit firmness is also considered as very important, which determines the evaluation of the consumers related to the quality of fresh lemons, but is also related to the potential storability during postharvest operations. In this report, GABA treatments (at 10 or 100 mM) did not impact any negative effect of firmness, although the 50 mM showed a significant reduction in fruit firmness at thee time of harvest. Information about preharvest GABA treatments on the quality of horticultural products is limited to cut flowers, although some evidence exists for when GABA is applied as postharvest treatments.

5. Conclusions

This is the first report that studied the effect of GABA preharvest application on citrus fruits. The application of GABA as preharvest treatments demonstrated a significant effect in enhancing the yield of 'Fino-95' lemons during two consecutive seasons (2019–2020 and 2020–2021), the most significant results being obtained for GABA at 100 mM. In addition, this GABA concentration did not impart any negative effect on the lemon quality attributes such as TSS, TA, and firmness. In future, the effect of GABA in other lemon cultivars such as 'Verna' and its role in the bioactive compounds and antioxidant enzymes requires further investigation. Thereafter, the possible role of preharvest GABA treatments on improving lemon quality during postharvest storage will provide a wide scenario of this natural elicitor compound.

Author Contributions: M.S. and D.V. conceived and designed the work; S.C., D.M.-R. and J.M.V. performed the field treatments; F.B. and J.M.V. performed most of the analytical determination, in collaboration with the other authors; M.S. and D.V. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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