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Research article

NUTRITIONAL QUALITY AND AGRONOMIC YIELD OF *Cucurbita moschata* HYBRID COMBINATIONS

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ABSTRACT

This study investigated the possibilities of improving the nutritional quality and agronomic yield of butternut squash (Cucurbita moschata) fruits through conventional breeding. The second objective was to assess the effects of high-temperature stress on yield- and quality-related parameters. The plant material comprised five parental genotypes and ten corresponding F_1 hybrids, obtained through partial diallel crossing. The nutritional quality and agronomic yield parameters were evaluated over two growing seasons characterized by distinct temperature regimes (temperate and high-temperature stress). The hybrids outperformed the parental lines in the fruit number and yield per plant, although they generally did not show similar improvements in nutritional quality. However, the wide ranges of variation in the carotenoid and sugar content observed among the hybrids indicate the potential for improving nutritional quality traits through breeding. High-temperature stress was predominantly associated with the decreased carotenoid content and increased sugar content in the fruits. Although the successful registration of new improved varieties and hybrids requires multi-year experimental data, the presented findings could provide a valuable contribution to butternut squash breeding for simultaneously enhanced agronomic yield and nutritional quality of fruits.

Key words:
butternut squash, carotenoid content, Cucurbitaceae, fruit flesh quality, high-temperature stress, vegetable breeding

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INTRODUCTION

The fruits of plants belonging to the genus *Cucurbita*, commonly referred to as pumpkins and squashes, play a significant role in both human and animal nutrition. Among the many species within this genus, *Cucurbita pepo* (oil pumpkins, vegetable marrows, zucchinis, and pattypan squashes), *Cucurbita maxima* (pumpkins), and *Cucurbita moschata* (butternut squashes) are of economic importance in agriculture. Each of these species encompasses numerous varieties and forms, categorized into distinct cultivar groups.
While pumpkin production in general remains substantial, butternut squash is less represented, though it may still contribute to the agricultural economy. Butternut squash is a valuable vegetable, with carotenoids as the principal compounds enhancing its nutritional quality. The consumption of carotenoid-rich foods has been associated with

improved immunity and a reduced risk of degenerative diseases. These biomolecules also help protect the skin from harmful UV radiation and possess anti-inflammatory properties. In addition to carotenoids, the nutritional quality and organoleptic properties of butternut squash are influenced by sugars, amino acids, minerals, and polyphenols. Pumpkins and squashes are low-acid foods, with a pH value close to neutral (7.0). Given its high nutritional quality, butternut squash can be considered a functional food. Its consumption supports digestive health, and allergic reactions are extremely rare. For these reasons, it is especially recommended in the diets of young children. Butternut squash is very low in calories, averaging about 40 calories per 100 grams of fresh fruit, making it an excellent addition to weight-loss diets. Though, in Serbia and the surrounding region, it is primarily used in sweet dishes such as pies, compotes, juices, and baby food. The most popular varieties are characterized by an orange-brown epidermis and are colloquially referred to as 'tamburica.' Presently, only two registered cultivars are listed in the Serbian database, and local producers often rely on saved seed for Enneb et al., 2021; Hosen et al., 2021; Hussain et al., 2021; Men et al., 2021).

This pilot study was undertaken to assess the possibilities of enhancing butternut squash quality and yield through conventional breeding. Although *Cucurbita* plants thrive in warm regions, prolonged periods of high or extremely high daily temperatures can reduce both fruit quality and yield. Therefore, the secondary aim was to examine the effects of high-temperature stress on fruit quality and yield parameters.

MATERIAL AND METHODS

The two-season results presented in this paper are part of an ongoing multi-year field trial aimed at improving the yield and quality of *Cucurbita* plants through conventional breeding. The trial is conducted at the Department of Vegetable and Alternative Crops, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia. The experimental plots are located at the Bački Petrovac field site (N 45°20', E 19°40', altitude 82 m) and are arranged in a randomized block design with replications. The main plot consists of a 20.0 m long row containing ten plants spaced 2.0 m apart, with rows planted 5.0 m apart. The sowing took place in the first decade of May, and the harvesting at the end of September. The plants were grown following the standard regional production practice, including the adequate autumn fertilization (16:16:16 NPK, 200 kg/ha), regular weed management, sprinkler irrigation, and plant protection as required. Accordingly, elevated daily air temperatures represented the primary environmental constraint affecting the plant growth and development. The 2020 season, with temperatures approximating the multi-year means, was classified as temperate, whereas the 2017 season, marked by an exceptionally high number of hot days, was designated a high-temperature stress season (Figure 1). The weather data were obtained from the Republic Hydro-Meteorological Service of Serbia (2024).

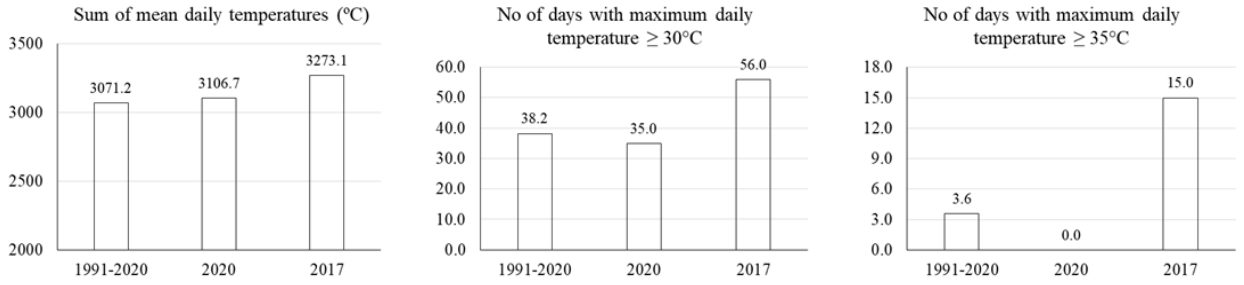

Figure 1. Air temperature regimes throughout *Cucurbita moschata* growing seasons of 2020 (temperate), 2017 (high-temperature stress), with multi-year (1991-2020) means (May-September)

The plant material consisted of five parental butternut squash (*Cucurbita moschata*) genotypes and their F1 hybrid offspring, obtained through partial diallel crossing (10 combinations in total). The crossings were performed in the 2016 season. The parental genotypes were chosen from the Institute's breeding collection and included 'Waltham Butternut', 'Butterbush Strain', 'Bush Butternut', and 'Menina Creme' (entered the collection as purchased commercial cultivar seed and maintained by controlled self-fertilization), as well as TJK 2006:263, a genotype originating from Tajikistan and obtained through the Germplasm Resources Information Network, courtesy of the Agricultural Research Service, United States Department of Agriculture (USDA, 2015).

The analyses of nutritional quality parameters were performed on ripe harvested fruits. The assessed fruit flesh quality parameters included dry weight (%), sugar content (mg/g fresh weight), protein content (mg/g fresh weight), carotenoid content (mg/kg fresh weight), refractive index, total soluble solids content (°Brix), and flesh juice pH.

The parameters of agronomic relevance comprised fruit weight (kg), number of fruits per plant, and yield per plant (kg). The methodology for chemical analyses was described in detail by Brdar-Jokanović et al. (2019).
Descriptive statistical parameters (mean, coefficient of variation, minimum, and maximum values) were calculated, and the least significant difference (LSD) test was used for means comparison.

RESULTS AND DISCUSSION

A partial diallel cross among five parental genotypes was conducted to evaluate the potential for improving butternut squash nutritional quality and yield through selective breeding. The cross resulted in ten F1 hybrid combinations. The fruit quality and yield parameters were assessed within the ongoing multi-year field trials to evaluate the environmental impacts and determine the stability of genotypes. As all recommended agro-technical practices were applied, high and extremely high air temperatures (daily maximum ≥ 30 and 35 °C, respectively) emerged as the primary uncontrolled environmental constraint. In this regard, the performances of the investigated parameters were compared across two growing seasons characterized by differing temperature regimes: 2020 (temperate) and 2017 (high-temperature stress).
As shown in Figure 1, the 2017 growing season was marked by a comparatively high sum of mean daily temperatures, an increased number of hot days, and a significant number of extremely hot days. While the 201.9 °C difference between the sum of mean daily temperatures recorded in 2017 and the multi-year mean does not necessarily lead to butternut squash crop failure, the same cannot be applied for the number of hot, and especially extremely hot days. The development of male flowers is promoted under high-temperatures, however heat shock induces pollen sterility. Conversely, female flowers may senesce without opening, and even when fruit set occurs, immature fruits are often aborted prior to maturation (Wien et al., 2002; Paris, 2016; Shehata and Abdelgawad, 2019). These physiological disruptions are expected to reduce the number of mature fruits per plant, thus lowering overall yield.
The two-year mean values for the investigated parameters along with the corresponding coefficients of variation, are presented in Table 1. These values generally fall within the ranges reported by other authors, e.g. Azevedo-Meleiro and Rodriguez-Amaya (2007), Jacobo-Valenzuela et al. (2011), Kim et al. (2012), and Men et al. (2021).
Regarding fruit nutritional quality, protein content and dry weight differed significantly between the parental and hybrid genotypes. For the other parameters differences were statistically insignificant. The highest coefficients of variation were calculated for the carotenoid and sugar contents. The values of the quality parameters were generally higher in the parental group. On the other hand, the hybrids achieved a significantly higher agronomic yield, primarily due to a higher number of fruits per plant, which was most likely a result of better pollination, fruit setting, and a greater proportion of unaborted fruits. This was rather expected as the butternut squash fruit yield improvements obtained through conventional selection breeding have been documented by several authors (Tamil Selvi et al., 2013; Kumar et al., 2022; Kouago et al., 2024). Although the two-year mean values of carotenoid and sugar content did not differ significantly between the parents and hybrids, the high coefficients of variation indicate a range that could be leveraged to improve quality. The potential of exploiting hybrid vigor to enhance the nutritional quality of *Cucurbita* plants has been discussed by Loy (2004), El-Tahawey et al. (2015) and Hosen et al. (2022).

Table 1. Nutritional quality and agronomic yield parameters (above and below horizontal line, respectively) in five parental and ten F1 hybrid butternut squash genotypes, two-year means

Parameters	Parents		F1 hybrids	
	Mean	CV (%)	Mean	CV (%)
Dry weight (%) *	14.9	20.2	12.4	13.8
Sugar content (mg/g)	31.6	30.2	29.7	37.2
Protein content (mg/g) *	3.4	9.7	2.6	23.0
Carotenoid content (mg/kg)	27.4	62.7	27.2	73.5
Refractive index	1.349	0.1	1.347	0.3
Total soluble solids content (°Brix)	10.9	9.9	9.4	24.5
pH	6.7	5.2	6.4	2.3
Fruit weight (kg)	1.4	41.9	1.6	32.9
Number of fruits per plant **	3.1	25.8	4.2	24.0
Yield per plant (kg) *	4.5	55.6	5.8	58.7

Legend: CV – coefficient of variation, *, ** – significant difference (P < 0.05, 0.01)

The values of the analyzed parameters for plants grown under contrasting temperature regimes are presented in Figure 2.

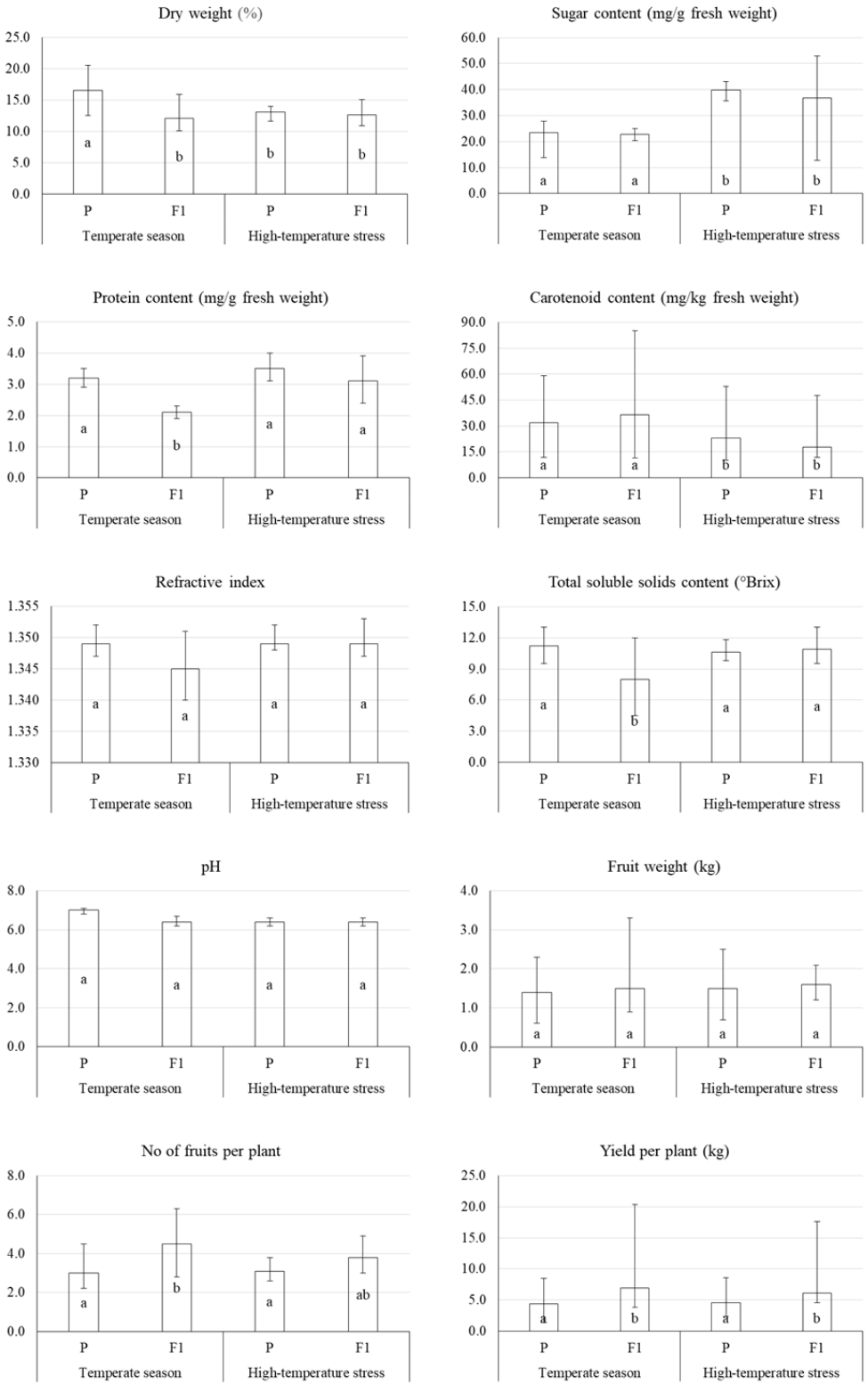

Figure 2. Butternut squash nutritional quality and agronomic yield parameters observed in two growing seasons with contrasting temperature regimes. P – parental genotypes, F1 – hybrids

Significant differences were observed between the two temperature regimes for several nutritional quality parameters. The most pronounced effects of high-temperature stress are observed as an increase in sugar content and a decrease in carotenoid content, a trend consistent across both the parental lines and hybrids. In contrast, the refractive index and pH were not significantly affected by the genotype or season. Similar patterns were reported in a study involving 40 *Cucurbita maxima* genotypes (Brdar-Jokanović et al., 2019).

Although elevated sugar content enhances fruit taste, future research should address the qualitative composition of sugars, given their role in plant responses to heat stress (Liu et al., 2013; Mathieu et al., 2018). Altered accumulation patterns of fructose and sucrose under high temperatures, observed in other agricultural plants (Cho et al., 2021), may also occur in butternut squashes. The considerable variation in sugar content among the hybrids highlights the potential for selection. The variation in carotenoid content is also wide, which applies to both parents and hybrids under both temperature regimes. While heat stress generally reduces the carotenoid accumulation, it is important to consider the stability of specific genotypes in this context. Multi-year experiments are essential to reliably identify and utilize such stable genotypes.

Regarding the parameters of agronomic importance, the hybrids exhibited significantly higher yield per plant compared to the parental genotypes, a trend consistent in both temperature regimes. However, the wide variation observed among the hybrids warrants further attention, particularly concerning the yield stability of individual genotypes. Since no significant differences in fruit weight were detected, the increased number of fruits per plant appears to be the primary contributor to the higher yields observed in the hybrids. Further research is necessary to determine whether this trait results from the enhanced pollen viability and/or increased plant vigor, which may enable the development and maturation of a greater number of fertilized female flowers.

CONCLUSION

The investigated *Cucurbita moschata* hybrids demonstrated a significant improvement over the parental plants in traits of agronomic importance, though they generally did not show similar advancements in fruit quality parameters. Nevertheless, the wide range of variation observed for carotenoid and sugar content in the hybrids suggests that there is still potential to improve these parameters through breeding. The most notable effects of high-temperature stress on butternut squash fruit flesh quality were reduced carotenoid content and increased sugar content. Further experiments are required to assess the stability of individual hybrids in terms of fruit yield and quality across different seasons.

Authors' Contribution: Conceptualization: M.B.-J.; Methodology: M.B.-J., B.K. and V.S.; Software: M.B.-J. and B.K.; Validation: M.B.-J., B.K., M.K., M.M. and V.S.; Formal Analysis: M.B.-J.; Investigation: M.B.-J., B.K., M.K. and M.M.; Resources: M.B.-J. and V.S.; Data Curation: M.B.-J.; Writing – Original Draft Preparation: M.B.-J.; Writing – Review & Editing: M.B.-J., B.K., M.K., M.M. and V.S.; Visualization: M.B.-J.; Supervision: V.S.; Project Administration: M.B.-J.. All authors have read and agreed to the published version of the manuscript.

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Data availability statement: The data supporting the findings of this study are available from the authors upon reasonable request.

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