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Scientific article

Heteromorphic, physiological and molecular characteristics of *Gazania spp* grow in North Coast of Egypt

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Abstract

Gazania (L.) is one of wild ornamental plant which has special architectures. It is a small genus of 16 species, belonging to the family Asteraceae (Formerly: Compositae). It is low-growing perennial herb, grow in clumps and can also form dense carpets covering large areas. *Gazania* has multiple genera and species with mutli-petals colors. The chlorophyll pigmentation was measured in leaves and petals of these specimens which show high significant difference. Genomics is the study of genomes, and it can be used to conduct research on genome evolution, genomic variations, gene regulation, and important biological mechanisms using detailed genome sequence information. The diversity of ornamental plants, as well as the slow progress of genome research on ornamental plants for a long time. RAPD-PCR was applied in the present study to estimate the genetic variation in these samples which resulted in a total polymorphism percentage of 42.36%. This polymorphism percentage lead to the variation in essential physiological pathways in *Gazania spp* and reflected on the multivariate petals color.
Keywords: Carotenoids, Chlorophyll, *Gazania krebsiana*, *Gazania rigens*, RAPD-PCR.

Resumo

Características heteromórficas, fisiológicas e moleculares de *Gazania* spp crescem na costa norte do Egito

Gazania (L.) é uma planta ornamental silvestre que possui arquiteturas especiais. É um pequeno gênero de 16 espécies, pertencente à família Asteraceae (Anteriormente: Compositae). É uma erva perene de baixo crescimento, cresce em tufos e também pode formar tapetes densos que cobrem grandes áreas. Gazania tem vários gêneros e espécies com cores de múltiplas pétalas. A pigmentação da clorofila foi medida nas folhas e pétalas desses espécimes que apresentam alta diferença significativa. Genômica é o estudo de genomas e pode ser usada para conduzir pesquisas sobre a evolução do genoma, variações genômicas, regulação gênica e mecanismos biológicos importantes, usando informações detalhadas da sequência do genoma. A diversidade das plantas ornamentais, assim como o lento progresso da pesquisa do genoma de plantas ornamentais há muito tempo. O RAPD-PCR foi aplicado no presente estudo para estimar a variação genética nessas amostras que resultou em uma porcentagem total de polimorfismo de 42,36%. Este percentual de polimorfismo leva à variação nas vias fisiológicas essenciais em *Gazania* spp e reflete na cor multivariada das pétalas.
Palavras-chave: Carotenóides, Clorofila, *Gazania krebsiana*, *Gazania rigens*, RAPD-PCR.

Introduction

Ornamental plants are economically important for the horticultural industry. They are sold all over the world as cut flowers and are used in gardening, landscaping, and floristry. Improved floral anatomy and morphology, new floral color, induced early flowering, enhanced fragrance or longevity, stress tolerance or disease resistance have all been used to introduce original traits of high commercial interest for producers and/or consumers through genetic modification of ornamental plants (Azadi et al., 2016; Darqui et al., 2017; Kishi-Kaboshi et al., 2018; Scaramuzzino, 2019; Boutigny et al., 2020). Ornamental plants affect impact on human life through their variety of shape, color, leaves, flowers, and scent fragrance (Abdulrazzaq, 2017; Dębicz et al., 2017).

Gazania is a flowering plant genus native to Southern Africa that belongs to the Asteraceae family. Throughout the summer, they produce large, daisy-like composite flowerheads in brilliant shades of multi-colors. It is an herbaceous perennial plant named for a flower shape resembling a medal They're commonly used as a drought- and salt-resistant groundcover. It is a type of annual flower

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that serves as a decorative element for public greens, gardens, and flower stands (Howis et al., 2009; Talebi et al., 2014; Zeng et al., 2016; El-Naggar et al., 2020).

Gazania rigens (L.) *Gaertn.* (Treasure flower) is a widely cultivated ornamental garden plant that has naturalised elsewhere. *Gazania* is an annual perennial that thrives in rock gardens and other hot, dry environments. Generic name *Gazania* came from the Greek 'Gaza' which refers to the rich color, variety, and availability of the plant. It grows as a low, ground-hugging ground cover with daisy-like flowers in bright yellow, orange, or red. At night and on cloudy days, flowers close shop. It's an excellent plant for preventing erosion in dry areas. It should be planted 12 to 18 inches apart to form a solid ground cover after a few months. The plant thrives in good soil, but it can also thrive in poor, sandy soil. Most annuals and perennials require more water than *Gazania*. The flowers prefer full sun and do not open fully on cloudy or rainy days. Disease can be spread by prolonged wet weather or poorly drained soil, killing parts of the plant. Removing the old blossoms may result in more blooms being produced (Reddy et al., 2014; Salehi Sardoei and Shahdadneghad 2014; Sabatino et al., 2019). *Gazania krebsiana* Less. is a grassland species that clings to the ground. It is one of the first plants to bloom in the spring, with small clumps of yellow or white flowers appearing between low grass tussocks or burnt stubble, or as leafless single flowers that appear to be stuck in the soil. Buttercup-yellow rays are frequently found in forms with dark spots at the base. This species is used in folk medicine and antibiotics (Tshabalala et al., 2016; Hlokoane 2021).

To develop new floral crops and breed new cultivars, numerous studies on plant genetic resources exploitation and ornamental plant breeding have been conducted. More than 6000 species were rated potential as ornamental plants after nearly ten years of investigation and evaluation of wild plant species and some cultivated species across many countries. Morphological traits and molecular markers were used to investigate the genetic diversity of some unique ornamental plants in China (including *Prunus mume, Chrysanthemum morifolium, Gazania spp., Paeonia spp., Rosa rugosa, Lilium spp., Lagerstroemia spp*., and others) (Zhang et al., 2012).

In this study, a classification of different *Gazania spp* was performed. This study based on morphological description of petals, pigmentation concetration, and total protein contents. The RAPD-PCR molecular marker was applied to estimate the genetic variation among these different species and forms.

Materials and Methods

Collection of Plant Materials

Five different *Gazania* forms were collected from Al-Zeraa Al-Bahary, Al-Hammam, North Coast, Egypt (km 69.150, Alex-Matrouh Costal Rd.). The latitudes of samples were recorded; latitude 30.862492 and longitude 29.311286. The specimens were collected from a scientific tripe at January, 2021. The collected samples have different petal patterns in color and shape.

Morphological Description

The collected *Gazania* samples were morphologically identified and described according to Boulos (2005) and Kadereit and Jeffrey (2007).

Estimation of Petals Pigmentation

According to Metzener et al. (1965), chlorophyll a, b, and carotenoids were measured in both the plantlet's fresh leaves and colored petals separately. In 5 ml of 85% acetone, a known weight of fresh leaves (0.5 g) was homogenized. The pigment-containing supernatant was made up to a definite volume (10 ml) with 85% acetone after centrifugation. At three wavelengths (452, 645 and 664 nm), the extract was compared to a blank of pure 85 percent aqueous acetone using a colorimeter. The concentration of chlorophyll a, b and carotenoids were calculated as µg g^{-1} leaf fresh weight using the following equations:

$$Chl.a = (10.3xA664) - (0.918xA645)$$

$$Chl.b = (19.7xA645) - (3.87xA664)$$

$$Carotenoids = (4.3xA452)x\big((0.0265xChl.a) + (0.426xAChl.b)\big)$$

where A= absorbance at different wave lengths.

After that, the fractions were calculated as mg g^{-1} fresh weight:

$$\frac{Fraction \times dilution}{1000} \text{ mg g}^{-1}$$

Estimation of Total Proteins

The total protein was extracted according to Bradford (1976) and some modification by Tawfik (2021) and Hussien et al. (2021) as follows: 0.5 g of leaves was weighted and grind well with 0.5 ml of [2x] Bradford reagent. Then vortex 10 min and centrifuge for 15 min at 24,104 g at 4 °C. The supernatant contained the total protein content of plant species. Finally, the protein concentration was estimated according to (Bradford, 1976) as follows: 0.1ml of supernatant was pipette into a test tube and 5ml of protein reagent was added, mixed and measured by spectrophotometer at wavelength 595 nm. The concentration of protein was determined from the protein standard curve (using bovine serum albumin). The concentration was calculated according to the following equation:

$$X = \frac{Y - 0.030}{0.007}$$

where X was the protein concentration (mg g^{-1}), and Y the absorbance (nm).

DNA Isolation and RAPD-PCR Bioassay

Edwards et al. (1991) used the cetyltrimethylammonium bromide (CTAB) method to isolate total genomic DNA from each *Gazania spp* of form. About 0.3 g of leaves was ground in 700 µl of 2% CTAB buffer and incubated for 30 min. at 65 °C with vertexing every 10 minutes. The supernatant was transferred into new Eppendorf tubes

after centrifugation at 12,000 rpm for 10 min. Each tube was filled with an equal volume of chloroform: isoamyl alcohol (24:1) and allowed to sit for 2 min. at room temperature before being centrifuged at 12,000 rpm for 10 min. at 4 °C. The upper aqueous layer was transferred to new tubes and 800 µl of absolute ice-cold ethanol was added before being left at -20 °C for about two hours. After that, centrifuge to extract DNA pellets and then wash them in 70% ice-cold ethanol. Finally, resuspend the pellets in 50 µl of Tris/EDTA buffer (TE) buffer and keep them at -20 °C till applying the RAPD-PCR reaction.

In this bioassay, ten RAPD decamer primers were used. Only six of them produced repeatable clear bands, Table (1). In a Biometra thermocycler, the RAPD-PCR reaction was carried out as follow; the reaction mixture containing 12.5 µl Taq master mix (COSMO PCR RED M. Mix, W1020300x), 2 µl genomic DNA, 1 µl each primer (Willowfort), and 9.5 l dd-H_2O in a total volume of 25 µl. The PCR reaction program was as follows; initial denaturation at 95 °C for 5 min, 35 cycles of denaturation for 30 s at 94 °C, annealing 30 s, and extension for 1 min at 72 °C. After that, one step of final extension at 72 °C for 10 min then cools at 4 °C. The amplified PCR product was run on 1.4% agarose gel compared to (New England Biolab, #N3232S) ladder".

Statistical Analysis

The gel electrophoresis images were divided into two categories: 1 'band present' and 0 'band absent.' Using Jaccard's similarity coefficient, a pairwise similarity matrix was created. Cluster analysis was performed to develop a dendrogram using the unweighted pair group method with the arithmetic averaging algorithm (UPGMA). Bio-Rad Quantity one (4.6.2) was used to perform these calculations (Shuaib et al., 2007). The data was subjected to an analysis of mean averages and standard deviations were calculated using Minitab 19.

Results

Morphological Description of *Gazania spp*

The different forms of *Gazania rigens* and *Gazania krebsiana* were collected from Al-Hammam region. However, they were collected from the same region; it was observed the different patterns of color and shape of their petals (Figure 1). Sample (1): it was identified as *Gazania krebsiana* Less. and has red petals with dark red central line and purple tips, it also known as "Copper King or Harlequin". Sample (2): it was identified as *G. rigens* and it has homogenous white petals with yellow ends. Sample (3): *G. rigens*, homogenous orange color. Sample (4): *G. rigens* var. *rigens*, orange petals with central red line. Sample (5): *G. krebsiana* Less., homogenous yellow color. All the petals have tapered end and black color at their base connected to the flower disc. The leaves of all forms are foliage leaves with same description of shape and color.

Table 1. Primer Data analysis of RAPD-PCR bioassay with different *Gazania spp*

No.	Primer name	Primer's sequence	GC%	Tm	Total bands	Total polymorphic bands	Polymorphism %
a	OPA-12	5'-TCGGCGATAG-3'	60	34	6	1	16.67
b	OPA-09	5'-GGGTAACGCC-3'	70	37.4	9	3	33.33
c	OPB-17	5'-AGGGAACGAG-3'	60	33.1	9	3	33.33
d	OPN-02	5′-AACCAGGGGCA-3'	70	36	6	2	33.33
e	OPN-04	5′-GGACCGACCCA-3'	80	38	8	5	62.5
f	OPZ-07	5'-CCAGGAGGAC-3'	70	34.6	4	3	75
Total					42	17	42.36

Figure 1. Different forms of *Gazania spp*.; *G. krebsiana* (1), *G. rigens* (1), *G. rigens* (3), *G. rigens* var. *rigens* (4), *G. krebsiana* (5).

Physiological Parameters of *Gazania spp*

Leaves and petals pigmentation concentrations and total protein contents were the physiological parameters measured for *Gazania spp.* (Figures 2 and 3). The different color of their petals comes from the different pigmentation expressed from variable proteins in the synthesis pathway of these different pigments.

Molecular Marker Bioassay

High molecular weight genomic DNA was isolated successfully from all the fresh and dried tissues samples. Ten RAPD primers were applied and only six of them gave clear and reproducible bands. RAPD reactions resulted in some polymorphic bands in many primers (Figure 4).

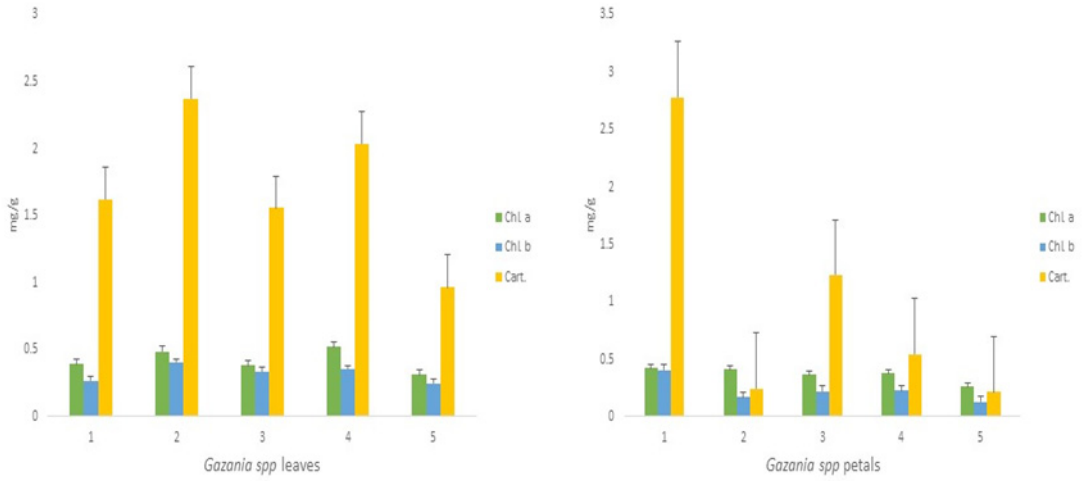

Figure 2. Different pigmentations' concentrations in leaves and petals of *Gazania spp.*

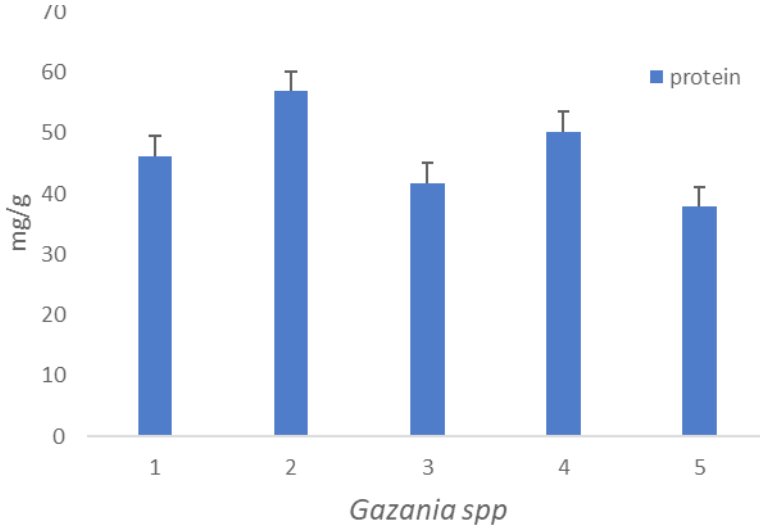

Figure 3. Total protein content in *Gazania spp.*

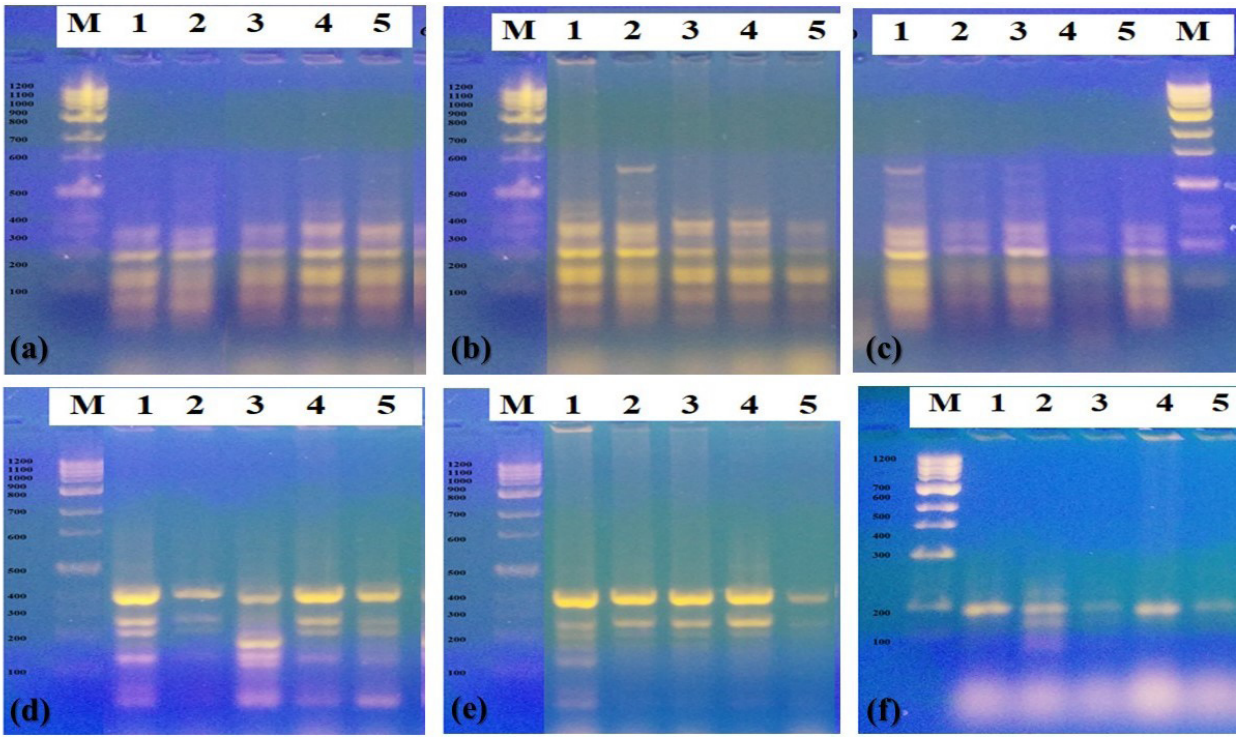

Figure 4. Fingerprint patterns generated using Gel banding pattern of RAPD-PCR (a) OPA-12 primer, (b) OPA-09, (c) OPB-17, (d) OPN-02, (e) OPN-04 and (f) OPZ-07 for *Gazania spp*.; 1 *'G. krebsiana'*, 2 *G. rigens*, 3 *G. rigens*, 4 *G. rigens* var. *rigens*, 5 *G. krebsiana*.

These bands ranged in size from 180 to 850 bp resulting in a total number of 42 bands with 17 polymorphic bands. The total polymorphism percentage resulted from all these primers were 42.36%, (Table 1). These polymorphic bands could be expressed into different proteins leading to different pigmentation. The total monomorphic bands resulted from the six primers were 25 bands. These monomorphic bands ensured the main ancestor of these species and they are closely related species.

The total phylogenetic tree was designed using CAP software (Figure 5). It is based on physiological parameters and total genetic variation similarity matrix. The tree illustrated that *G. krebsiana* was separated in clade away from the other forms. Where, *G. rigens,* sample (2), *G. rigens* var. *rigens,* sample (4) and sample (5) were closely related to each other's.

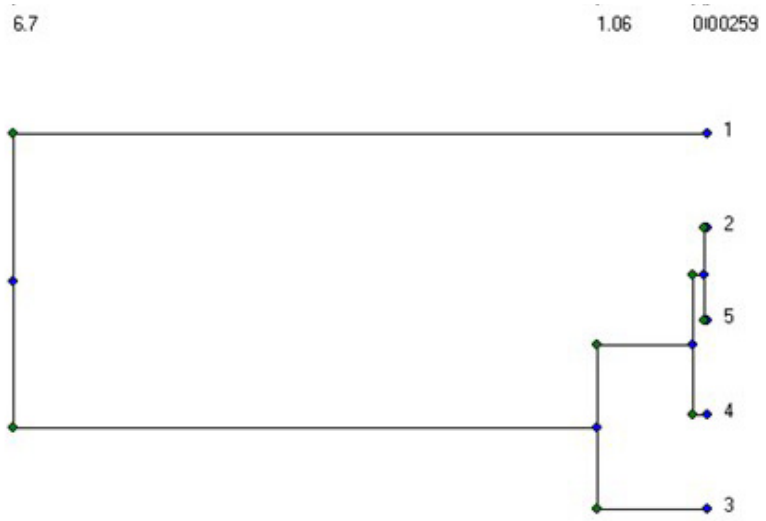

Figure 5. Phyllogenetic tree of *Gazania spp* based on physiological and molecular data based on Figure 1; 1 '*G. krebsiana'*, 2 *'G. rigens'*, 3 *G. rigens'*, 4 *'G. rigens* var. *rigens'* and 5 '*G. krebsiana'*.

Discussion

The variation in petals color and shape could be due to hybridization among the different Gazania species. These findings were confirmed by Kadereit and Jeffrey (2007) who stated that "Most common are hybrids originating from *Gazania krebsiana*, *G. linearis* and *G. rigens*, which are cultivated all over the world".

Another explanation of color variation could be due to soil constituents and how plant uptake the nutrients and minerals from soil. This phenomenon was explained by Hassan et al. (2015) "*This kind of soil spread in the northern western coast from Alexandria to the west limits of Egypt and in lot of the Arab countries, especially located in Mediterranean basin. Soil conditioners vary greatly in their composition, application rate, and mode of action. Several natural materials have been found to improve various physical properties of the calcareous soil such as compost, sand and others*."

The petals are usually orange, yellow, or white, but bronze or pink can also be seen. They are frequently brown at the base, forming an attractive ring in the center. In the dark, the flowers close and open again with the first rays of the sun. With a red stripe dissipating over each deep yellow petal, Daybreak Red Stripe' is a truly unique variation of this *Gazania spp*. When the flowers open, this creates the startling effect of a radiating sun (Salehi Sardoei and Shahdadneghad 2014). Also, Talebi et al. (2014) confirmed the variation in flower colors and mentioned that "increasing of ornamental performance is a major concern, especially in harsh urban condition". Howis et al. (2009) also, mentioned that the taxonomy of *Gazania* is confusing in both wild and cultivated species.

The description and identification of *G. krebsiana* agreed with Batten (1988) who stated that "The buttercup-yellow rays often occur in forms having dark spots at the base. The showy flowers open only in strong sunlight, closing with fading light and re-opening the following day. This plant is perennial and grows from a woody rootstock. The leaves are tufted and linear in shape, white-felted on the underside, and exuding milky latex when damaged".

Total carotenoids increased with increase of total proteins in the different *Gazania spp*. According to Bahari et al. (2013), the highest value of total carotenoids was achieved by using amino acid treatments on *Triticum aestivum* L. Nia et al. (2015) also stated that amino acids are necessary components of all cells. They participate in both primary and secondary metabolic processes associated with plant development and stress responses, in addition to protein synthesis.

Amino acids are necessary components of all cells. They participate in both primary and secondary metabolic processes associated with plant development and stress responses, in addition to protein synthesis. Glutamate, glutamate, aspartate, and asparagines, for example, serve as nitrogen pools and transporters while also balancing the carbon/nitrogen ratio. Muller et al. (1995) found that other amino acids like tryptophan, methionine, proline, and arginine contribute to plant tolerance to biotic and abiotic stresses, either directly or indirectly, by serving as precursors to secondary compounds and hormones. Amino acids and phenolic acids applied to the leaves of *Ammi visnaga* (L.) Lam. plants improved growth and phytochemical traits such as pigment content (Talaat et al., 2013; Nia et al., 2015).

In many plant species, DNA analysis has become a standard method for estimating genetic diversity. Because of this, Prakash and van Staden (2007); Tan and Yiap (2007) recommend optimising DNA isolation protocols as a first step in molecular analysis of medicinal plants (2009). The RAPD method was used to estimate genetic diversity among *A. montana* genotypes. A technique known as randomly amplified polymorphic DNA identified a large number of polymorphic products. Kizhakkayil and

Sasikumar (2010), Okoń and Surmacz-Magdziak (2011) and Okoń et al. (2014). Zheng et al. (2021) mentioned that molecular markers were applied to estimate the genetic variation among Asteraceae genera.

Conclusions

Ornamental plants, which are an important part of agriculture and horticulture, are important for beautifying and improving people's living environments, cultivating human sentiment, and promoting agricultural structural changes. Gazania is an important ornamental genus with a wide range of petal colors. These variations in the physiological measurements come from the genetic variation in DNA contents, with percentage of 42.36%. All of these parameters reflected on morphology of petals shape and color patterns. So, the different patterns of the collected samples led to differentiation in classification of *Gazania* specimens.

Author Contribution

MSAS: Conceptualization, funding acquisition, resourses. **ET:** Formal analysis, investigation, original draft preparation, text review and editing, funding acquisition, resourses.

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