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[54]	GERANIUM	PLANT "CALYPSO"
127	QT:T/V3: 11 OTAT	LTWILL CUTILDO

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[57]

ABSTRACT

This invention relates to a new distinct cultivar of geranium, substantially as illustrated and described, characterized as being particularly well adapted to both commercial greenhouse production and garden performance, and possessing unique flavonol and anthocyanin profiles, double flowers and clear floral color.

3 Drawing Sheets

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The present invention relates to a new and distinct cultivar of geranium *Pelargonium*×hortorum called "Calypso". The cultivar is particularly well adapted to both commercial greenhouse production and garden performance. The novel characteristics of this cultivar 5 are double flowers and clear floral color. The cultivar is further characterized by unique biochemical fingerprint profiles.

The cultivar was developed from an organized, scientifically designed breeding program carried out at the 10 Department of Horticulture, The Pennsylvania State University, University Park, PA 16802 and specifically resulted from selection of the progeny of the selfing of geranium cultivar "Graeffin Mariza". The selection was asexually propagated by cuttings and the reproductions 15 ran true.

With reference to the detailed description of the cultivar which follows, the test plant was grown in a glass greenhouse in full natural light, at a night temperature of 60° F., and a day temperature of 75° F. Soilless medium was fertilized constantly with 300 ppm N-K. Color readings were taken under cool white fluorescent light at 220 foot candles and color identification was by reference to The Royal Horticultural Society Colour Charts, except where common terms of color definition 25 are employed.

DESCRIPTION OF THE FIGURES

FIG. 1 illustrates in color the cultivar including foliage and flowers.

FIG. 2 illustrates the anthocyanin profile obtained from HPLC. Quantitative values are found in the tables. Analyses included a single peak that represented both pelargonidin and petunidin 3,5-diglucosides. Corrections were made in accompanying tables.

Peak No.

- 1. Delphinidin 3,5-diglucoside
- 2. Cyanidin 3,5-diglucoside
- 3. Pelargonidin 3,5-diglucoside
- 4. Peonidin 3,5-diglucoside
- 5. Malvidin 3,5-diglucoside

FIG. 3 illustrates the flavonol profile obtained from HPLC. Quantitative values are found in the tables.

Peak No.

1. Quercetin 3-rhamnosylgalactoside

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- 2. Quercetin 3-rutinoside
- 3. Quercetin 3-galactoside
- 4. Quercetin 3-glucoside
- 5. Kaempferol 3-rhamnosylgalactoside
- 6. Kaempferol 3-galactoside
- 7. Kaempferol 3-rutinoside
- 8. Kaempferol 3-glucoside; Kaempferol 7-glucoside; Quercetin 3-rhamnoside
- 9. Kaempferol 3-xyloside
- 10. Kaempferol 3-arabinoside
- 11. Kaempferol 3-rhamnoside

THE PLANT

⁵ Classification:

Botanical.—Pelargonium \times hortorum.

Tradename. — #712 (80-205-3) = "Calypso".

Form: Semi-dwarf; free basal branching; comparatively compact growth habit; flowers close to foliage; free flowering; early.

Height: 24.0-30.0 cm.

Growth: Relatively short internodes, self branching from base; faster growth than standard.

Leaves:

Size.—8.0–14.0 cm.

Shape.—Reniform, variously lobed.

Margin.—Crenate.

Texture.—Pubescent, dull; veins recessed and prominent.

Color.—Top: Dark zone band; Fan 3, yellow-green group 143-A (RHSCC); Fan 3, yellow-green group 144-A (RHSCC). Bottom: Fan 3, green group, 143-C (RHSCC).

Ribs and veins.—Palmate veins.

Petioles: Fan 3, green group, 143-C (RHSCC). Stem:

Color. —Same as petioles. Internodes.—2.0-3.0 cm.

THE BUD

Shape: Upright, hemispherical. Size: 2.0-3.0 cm diameter.

INFLORESCENCE

Blooming habit: Continuous, upright, double, non-shat-tering, hemispherical.

Size: 11.0–14.0 cm across.

Borne: Umbel; florets on pedicel; pedicels on peduncle.

Florets:

Forms.—Petals irregular, twisted and upright, full double.

Color.—Top: Variable with inner "V" of darker color and outer "V" of lighter. Inner 'V':=Fan 5 1, red group, 55-A (RHSCC). Outer 'V':=Fan 1, red group, 52-C (RHSCC). Base 'V':=Fan 1, red group, 41-C (RHSCC). Bottom of Petal: Quite variable; top half mottled=Fan 1, red group 49-A and C (RHSCC); base=Fan 1, red group, 10 41-C.

Petals.—20-25 (including petaloids) irregular, twisted and upright; reflexed.

Size. -3.0-4.0 cm.

reflexed.

Petaloids:

Quantity.—Not distinguishable from petals.

Shape.—Not distinguishable from petals.

Color.—Not distinguishable from petals.

Pedicel: 4.0-5.0 cm in length.

Color.—Fan 3, green group 143-C (RHSCC).

Peduncle: Arises from node; opposed to leaf petiole.

Length.—20.0-24.0 cm.

Color.—Fan 3, yellow-green 143-C 25 group (RHSCC).

Persistence: Persistent, non-shattering.

Disease resistance: Not known; favorable in outdoor trials.

Lasting quality: Excellent; 3 weeks or longer.

REPRODUCTIVE ORGANS

Stamens: 2-5.

Anthers.—Some are fertile; some are not; some are partially developed; tan or light brown in color. 35 Filaments.—Flat or semi-flat, some ribbon-like, some petal-like; 0.5 cm long.

Pollen.—Light orange.

Staminodes. —Up to 5 in number; some fused and some petal-like.

Pistils:

Number.—1 with 5-parted stigma.

Length.—1.0 cm.

Stigma.—5-parted; reddish-purple, reflexed.

Style.—1: 2.0 mm long; reddish-purple.

Ovaries: 1: 6.0 mm long; green; pubescent; 5-lobed. Fruit: None.

BIOCHEMICAL PROFILES

In recent years, biochemical analysis has played an 50 increasing role in plant systematics and taxonomy. In order to further characterize the cultivar, flavonols and anthocyanins were extracted from the florets and subjected to analysis by high pressure liquid chomatography (HPLC). Background information supporting the 55 validity of the HPLC technique can be found in an article by Asen & Griesbach ("High Pressure Liquid Chromatographic Analysis of Flavonoids in Geranium Florets as an Adjunct for Cultivar Identification", S. Asen and R. Griesbach, J. Amer. Soc. Hort. Sci. 60 108(5):845-850 (1983)), the contents of which are incorporated herein by reference. Briefly, the method for performing the analysis was carried out as follows:

Flavonoid extraction.

The sample size for flavonoid identification consisted 65 of the petals from six florets just after anthesis. Three different samples were collected from each cultivar and handled separately for analysis. The petals were

weighed, ground in 20 ml of 1% HCl-MeOH with a mortar and pestle, filtered through one layer of Whatman #1 filter paper, and washed with 1% HCl-MeOH. The volume was adjusted to 90 ml and 2-15 ml aliquots were removed for the analysis and handled separately. Each aliquot was taken to dryness at 40° C. in vacuo. All traces of HCl were removed by azeotropic distillation with MeOH. One of the dried extracts was reconstituted in 2 ml of 1% HCl-MeOH and was used for anthocyanin analysis. The other was reconstituted in 2 ml of MeOH and was used for flavonol analysis. Each sample was stored at -34° C. until analyzed.

HPLC.

Samples were analyzed on a Waters High Perfor-Texture and appearance.—Irregular surface, full 15 mance Liquid Chromatograph equipped with an automatic injection system (Waters Assoc. Wisp 71OA), dual pumps (Waters Assoc. Model 6000A), solvent programmer (Waters Assoc. Model 600), data module (Waters Assoc.), variable wavelength detector (Waters Assoc. Model 480), and a C_{18} column (25 cm \times 0.46 cm and 5 µm particle size, Supelco).

Most of the flavonol compounds were separated by a linear gradient of 8% to 23% pump B over 55 min (pump A=1% triethylamine buffered to pH 3.0 with H_3PO_4 (TEAP); pump $B = CH_3CN$) at a flow rate of 1.2 ml/min and a chart speed of 0.5 cm/min. Detector was at 340 nm.

The anthocyanins were resolved by a linear gradient $_{30}$ of 30% to 50% pump B over 40 min (pump A=1.5%) H_3PO_4 ; pump B=20% HOAc +25% CH₃CN +55% of 1.5% H₃PO₄) at a flow rate of 1.0 ml/min and a chart speed of 0.5 cm/min. Detection was at 546 nm utilizing a fixed wavelength detector.

The flavonoids were quantified by injecting standards and comparing their peak areas with those from the plant samples. The results are expressed as µg of flavonoid/g fresh weight of plant material.

Results

Chromatographic profiles for anthocyanins and flavonols are presented in FIGS. 2 and 3, respectively; quantification of these profiles by comparison to standards is presented in Tables 1 and 2, respectively.

The anthocyanins petunidin and pelargonidin 3,5diglucoside were not resolved by the solvent system used. Past research has shown only negligible amounts of petunidin 3,5-diglucoside to be present in geranium florets compared to pelargonidin 3,5-diglucoside. In light of this, the peak corresponding to petunidin and pelargonidin 3,5-diglucoside was quantified as pelargonidin 3,5-diglucoside.

Kaempferol 3-rhamnoside could not be quantitated for several cultivars and is designated as ND (not determined). The chromatograms showed a small, wide peak in the region of elution for this compound. If a substantial amount of this compound were present, a distinct peak appeared but minute quantities, if present, were masked.

Other barriers to quantitation of several flavonols existed. Kaempferol 3-glucoside, kaempferol 7-glucoside, and quercetin 3-rhamnoside all had the same retention time under these conditions. If these compounds are needed to distinguish between cultivars, they would have to be separated by other solvents or column types. Quercetin 3-xyloside appeared in several of the comparisons, but standards were not available to quantify this compound.

TABLE 1

An	thocyanin con	centration	in petals o	of geranium	florets	<u> </u>	
	μg an	thocyanidir	1 3,5-diglu	coside/g fi	esh wt.		_
Cultivar	Delphinidin	Cyanidin	Pelar- gonidin	Peonidin	Mal- vidin	To- tal	5
712	z	27	595	505	tγ	1132	•

 z_{-} = not detected

 $y_t = trace < 10 \mu g$.

What is claimed is:

1. A new distinct cultivar of geranium, substantially as illustrated and described, characterized as being particularly well adapted to both commercial greenhouse production and garden performance, and possessing unique flavonol and anthocyanin profiles, double flowers and clear floral color.

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			Flavon	ol cond	entration	in petals	of geran	ium flore	ts		
	μg/g fresh wt.										
Cultivar	Qu3- ^z rhagal	Qu3- rut	Qu3- gal	Qu3- glu	Km3- rhagal	Km3- gal	Km3- rut	Km3- xyl	Km3- arab	Km3- rha	Total
712	ŧУ	ŧ	t	15	19	70	84	10	64 .	NDx	277

^zAbbreviations: Km = Kaempferol; Qu = Quercetin; arab = arabinoside; gal = galactoside; glu = glucoside; rha = rhamnoside; rhagal = rhamnosylgalactoside; rut = rutinoside; xyl = xyloside.

 $y_t = trace < 10 \mu g$.

 ^{x}ND = not determined.

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