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The Relationship between Graft Compatibility and Cyanohydrin glycoside type and content in *Passiflora* species

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ABSTRACT

Production Passiflora edulis f. edulis Sims. (purple passion fruit) in Kenya is limited by the root fungus Fusarium. Some ornamental and wild Passiflora species have been found to be Fusarium-resistant though not compatible with Passiflora edulis f. edulis. This experiment was conducted to determine; graft compatibility between Passiflora edulis f. edulis scions and eight (P. altinis. P. biflora, P. caerulea, P. citrina, P. edulis, P. incarnata and P. yukatanensis) Passiflora species of two subgenera all cleft grafted and cyanohydrin glycoside type and content present in the foliage, stems of rootstalks and scion. Cyanohydrin glycoside analysis was carried out using a colorimetric assay of frozen stem and leaf samples. Passiflora incarnata and P. caerulea both members of the Passiflora subgenera had high percent (> 89%) graft success with P. edulis f. edulis scions. Species within the same subgenera (P. incarnata, P. caerulea and P edulis) and having the lowest concentration < 2.0 µmols amygdalin equivalents/g fresh weight of cyanohydrin glycoside had the greatest grafting success. Only P. edulis had type IV cynohydrin glycoside while P. altinis. P. biflora, P. caerulea, P. citrine and P. incarnata had type II cynohydrin glycoside.

Key words: *Graft compatibility; Passiflora genera; Cyanohydrin glycoside*.

INTRODUCTION

Passion fruit (*P. edulis* f. *edulis*) is rich in vitamins A (700 I.U/100g), riboflavin (0.13 mg/100g), niacin (1.5 mg/100g) and ascorbic acid (30 mg/100g), proteins (2.2 g/fruit), and minerals potassium 348 mg/100g), sodium (28 mg/100g), iron (1.6 mg/100g), phosphorous (64 mg/100g) and calcium (13 mg/100g) [14]. It also has several industrial uses (juice, pectin, oil, extraction and cattle and poultry feed preparation). For several years, passion fruit growing has been declining in many parts of the world due to *Fusarium* wilt infection and the passion fruit woodiness disease complex [3]. Resistant rootstocks are needed because *Fusarium* wilt control using methyl bromide fumigation is now banned world wide because of its greenhouse gas effect. Also, fumigation is not economically feasible for small-scale producers especially in Kenya. Currently, yellow passion fruit seedlings are used as rootstocks because they offer short term resistance to *Fusarium* wilt. Resistance to *Fusarium* wilt has been reported in a few ornamental species within the *Passifloraceae* family [15].

Breeding for new resistant rootstocks is being done in Australia [20]. Important results of breeding for resistance are the hybrid purple passion fruit which show considerable *Fusarium* wilt resistance. However, reports of new virulent *Fusarium* races appear within a few years after commercialization of resistant cultivars [1]. Other methods of control that are being tested include use of antagonistic micro-organisms (*Antagonistic Fusarium*) [4] and soil

nutrition. Soil nutrition involves the use of nitrogenous fertilizers with an aim of enhancing plant growth while the use of antagonistic microorganisms involves the use of beneficial microorganisms isolated from suppressive soils. Since these micro-organisms suppress the development of the pathogenic types, they represent an alternative method of managing the populations of pathogenic *Fusarium* types in soil [1]. Unfortunately, this method has been successful only in greenhouse environments with crops grown in soil-less substrates in containers. Current research in Kenya is geared towards finding a control method that can be used under field conditions. Possible methods include the use of soil amendments such as liming and finding alternative *Passiflora* species that are both compatible with the purple passion fruit and tolerant to *Fusarium* wilt [15]. Grafting aimed at taking advantage of the *Fusarium* resistant passion fruit species is an established cultural practice in many fruits. Some ornamental and wild *Passiflora species* have been found to be *Fusarium* resistant though not compatible with *Passiflora edulis f. edulis*. Several grafting techniques have been used in other crops. These techniques include whip, splice and cleft grafting. The cleft grafting method is the oldest and the most widely used method of grafting [10]. Cleft grafting is popular with passion fruit propagators because it gives excellent results. It is also relatively inexpensive and simple to make. Compatibility refers to the ability of the scion and the rootstock to join and grow as one plant [10]. Usually, incompatibility results when the scion and rootstock are distantly related genetically. Incompatibility between *Passiflora edulis f. edulis* and other ornamental and wild *Passiflora* species that been found to be *Fusarium*-resistant has been speculated to be caused by the type and content of the cyanohydrin glycoside compounds in the plant tissues. There are four common structural types of cyanohydrin glycoside compounds in the *Passiflora* genera: cyanohydrins with either a rare sugar residue, a sulfate group, or additional oxygenetion of the cyclopentane ring, linamarin or prunacin [11].

MATERIALS AND METHODS

2.1. Plant material

Plant material: Eight *Passiflora* species (*P. altinis, P. biflora, P. caerulea, P. citrina, P. edulis, P. incarnate, P. suberosa* and *P. yukatanensis*) were obtained from a commercial source (Glass House Works, Athens, OH). The plants were potted in one gallon containers and grown in a glass glazed greenhouse. The substrate used was Metro Mix 360 (Scotts-Miracle Gro, Maryville, OH). Irrigation with 200 mg/L of 20N-12.4K-4.5P water soluble fertilizer (Peters fertilizer, Scotts Miracle-Gro, Marysville OH) was carried out approximately 3 times per week. When the vine length of the shortest species was 20 cm (approximately eight weeks after potting), the vines were cut back to 15 cm length above the substrate surface. Then *P. edulis* scions 8 cm long with three-to-five buds were grafted on to each rootstock. Typically the diameter of the rootstock stem matched the diameter of the scion. On each rootstock, two graft combinations were made on separate branches. *P. edulis* scion was grafted onto the rootstock and a self-graft where the scion and rootstock were of the same species was made. There were three plants in each of the three replicates per graft combination. The 8 cm scions were sexually mature and obtained from the stock plants from which the cuttings were made. All grafts were cleft grafts. Before grafting, all the leaves were removed from the scion and after grafting the plants were covered with clear 16 x 12 cm polythene bags. The lower side of the bags was tied below the graft union with a rubber band. After two weeks, the bags were removed. Five weeks after grafting data on number of successful grafts was collected. At this time one hundred grams of leaf and stem fresh weight was collected from the scion and rootstalk of each plant. The leaves and stems were placed in plastic bags, sealed and frozen at -10^0 C until analyzed. Analysis was carried out at The Ohio Agricultural Research and Development Center in Wooster, Ohio. Spinach in polypropylene bags was purchased at a local grocery for use as a negative control.

2.2. Colorimetric assay

Frozen plant samples were divided into two 30±0.1 g extraction replicates; replicate weights were adjusted for species with limited supply. Replicates with similar portions of stem and leaf tissue were placed in a glass blender chamber containing 100 ml of 0.1M phosphoric acid, capped, pulsed until liquefied, then homogenized further at high speed from the predominance of fibrous material via gravity filtration using Spectra/Mesh woven polypropylene filter disks, transferred to large screw-capped test tubes and temporarily stored on ice until assayed for cyanohydrin glycosides. These samples were light green in color, opaque and contained suspended cellular material. The chloramines T assay procedure of Cooke [6]; [7] was used with modifications. Briefly 1.5 mL of sodium phosphate buffer (pH 6.0) and 0.5 mL of each of the two non-specific glycoside enzymes, β-glycuronidase from Helix aspera (snail gut enzyme in phosphate buffer-pH 6.8; 1925.5 IU/mL; [13]; [18]were added to aid inherent, cyanohydrin compound-specific glycosides present in the extract or replace them if not. After addition of enzyme, the tubes were capped and incubated in a water bath at 30^0 C for 15 minutes. After incubation, reactions

were stopped by addition of 1 mL of NaOH, tubes were recapped and placed in an ice bath for 10 minutes. The colorimetric procedure was initiated after removal from ice bath by adding 4 mL of phosphate buffer (pH 6.0) and 0.5 mL of the oxidizing agent. A barbituric acid-pyridine reagent was added [12] [3]. To prepare this reagent, 3 g of barbituric acid was dissolved in approximately 20 mL of boiling DDH_2O and stored in actinic glass. Exactly 1.5 mL of the barbituric acid-pyridine reagent was added to each sample and the samples were recapped. Color development was allowed to proceed for 60 min in the dark. Prior to spectrophotometry, the samples were clarified using 0.45 micron nylon syringe filters. The resultant red violet color (indicating the presence of cynide) was read at 575 nm against a phosphate buffer (pH 6.0) blank in a spectronic 20 using glass cuvetts. Absorbance units were transformed to umols of amygdalin equivalents on a standard curve (Y = 3.1789x + 0.036; correlation 0.996).

2.3. Data analysis

Data was subjected to multivariate analysis of variance test (MANOVA) using the GLM procedure within the SPSS the version for personal computers (SPSS 15.0, SPSS Inc, University of Chicago). Means were separated using Waller-Duncan test at p = 0.05 level of significance.

RESULTS

3.1. Cyanohydrin glycoside type and content

All *Passiflora* species had some cyanohydrin glycoside (Table 1). The species (*P. incarnata, P. caerulea and P edulis*) with the lowest concentration of cyanohydrin glycosides (μmols amygdalin equivalents per g fresh weight) had the greatest grafting success except for *P. biflora* (Table 2). The greatest scion growth rate was in graft combinations where the scion and rootstalk belonged in the same subgenera and both had less than 2 μmols amygdalin equivalents per g fresh weight of cyanohydrin glycosides (Table 2).

Pasiflora species within the *Decaloba* subgenera had either type I or a mixture of type I and type II cynohydrin glycoside while those species within the *Passiflora* subgenera had mainly low concentrations < 2 μmols amygdalin equivalents per g fresh weight type II with only *P. edulis* having 0.007 μmols amygdalin equivalents per g fresh weight type IV cynohydrin glycoside (Table 2).

3.2. The relationship between subgenera, the type and content of cyanohydrin glycoside, grafting success, scion growth rate and yield

Generally there appeared to be a relationship between subgenera, the type of cyanohydrin glycoside, grafting success and scion growth rate (dry weight accumulation/day) (Table 2). *Passiflora edulis* having type IV cynohydrin glycoside when self grafted had a high grafting success and a corresponding high scion growth rate five weeks after grafting (Table 2). Species with low cyanohydrin glycoside and belonging in the same subgenera as the scion had high grafting successes and subsequent high scion growth rate.

Ten weeks after grafting scions on *P. edulis* self grafts had the highest leaf numbers while all other scions on rootstocks belonging in the decaloba subgenera were stunted (Table 3). Scions on rootstocks belonging in the *Passiflora* subgenera but having low concentrations of type II cyanohydrin glycosides had small but more leaves compared to those on rootstalks in the same subgenera but having higher concentrations of type II cyanohydrin glycoside. Scion length, leaf number and fruit number one year after grafting was highest in *P. edulis* self grafts (Table 4).

DISCUSSION

Apart from the genetic differences, the most important causes of incompatibility are the physiological and biochemical differences between the rootstock and scion [10]. Spencer *et al* [19], Olafsdottir *et al* [16] [17], Jaroszewski *et al* [11] and Clausen *et al* [5] showed that cyclopentanoid cyanohydrin glucosides (cyanohydrin glucosides) are synthesized by all genera in the family Passifloraceae. They also reported that the type of cryohydrin glucosides produced were distinct at subgenus level. These results were consistent with the present findings.

According to Jaroszewski *et al*. [11], *P. caerulea* and *P. incarnata* synthesizes type II cryohydrin glucosides. Also, *P. muraja*, *P. citrina* and *P. mixta* synthesizes type I and II. In the present study *P. citrina* was found to have type II cryohydrin glucosides. Since one of the main phytochemical difference in the genus *Passiflora* is caused by cryohydrin glucosides [16] [17] and incompatibility is associated with the presence of different phytochemicals in the scion and stock [2]; grafting success in *P. incarnata* and *P. caerulea* both species with type II cryohydrin

glucoside as opposed to type IV cryohydrin glucoside found in the scion suggested that cyanohydrin glucosides produced by the rootstock was tolerated by the scion. Moreover, it has been reported that chemical treatments that reduce the amount of the cyanohydrin glycoside reverse the development of incompatibility and graft combinations producing relative equal amounts or types of cyanohydrin glycosides are compatible [9].

Table 1: Colorimetric assay of cyanohydrin glycoside content in the aerial portions of eight *Passiflora* spp. and spinach (*Spinaceae oleraceae*)

Sample	Mean ± S.E. (µmols amygdalin equivalents/g fresh weight)
Spinach[z]	-0.07 ± 0.00[y]
Passiflora spp.	
P. altinis	2.32 ± 0.10
P. biflora	0.27 ± 0.03
P. caerulea	1.52 ± 0.12
P. citrina	2.18± 0.03
P. edulis	0.07 ± 0.06
P. incarnate	1.58 ± 0.02
P. suberosa	7.23 ± 0.28
P. yukatanenesis	1.33 ± 0.09

[z]*Spinach analyzed as a control.*
[y]*Value is essentially zero.*

Table 2: Subgenera, grafting success, amygdalin equivalents/g fresh weight, scion growth rate five weeks after grafting and type of cyanohydrin glycoside in eight *Passiflora* species

Species	Subgenera	Grafting success (%)	µmols amygdalin equivalents/g fresh weight	Scion dry weight accumulation (g/day)	Type of cyanohydrin glycoside (s)
P. altinis	*Passiflora*	23.3d	2.32b	0.01c	II
P. biflora	*Decaloba*	66b	1.27c	0.03bc	II
P. caerulea	*Passiflora*	89a	1.52c	0.77a	II
P. citrina	*Decaloba*	66b	2.18b	0.02c	II
P. edulis	*Passiflora*	100a	0.007d	0.81ab	IV
P. incarnata	*Passiflora*	100a	1.58c	0.52abc	II
P. suberosa	*Decaloba*	43.3cd	7.23a	0.07a	I,II
P. yukatanenesis	*Decaloba*	56bc	1.33c	0.03bc	I,II

Table 3: Subgenera, grafting success, amygdalin equivalents/g fresh weight, scion leaf number and total leaf area (cm^3) in eight *Passiflora* species ten weeks after grafting

Species	Subgenera	Grafting success (%)	µmols amygdalin equivalents/g fresh weight	Scion leaf number	Total leaf area (cm^3)
P. altinis	*Passiflora*	23.3d	2.32b	2c	52.78cd
P. biflora	*Decaloba*	66b	1.27c	2.5c	34.11de
P. caerulea	*Passiflora*	89a	1.52c	5b	81.89b
P. citrina	*Decaloba*	66b	2.18b	2c	30.22e
P. edulis	*Passiflora*	100a	0.007d	8a	511.99a
P. incarnata	*Passiflora*	100a	1.58c	5.5b	67.33bc
P. suberosa	*Decaloba*	43.3cd	7.23a	2c	24.36e
P. yukatanenesis	*Decaloba*	56bc	1.33c	2c	32.78e

Table 4: Subgenera, scion length, leaf number and fruit number in eight grafted *Passiflora* species one year after grafting

Species	Subgenera	Scion length (cm)	Scion leaf number	Fruit number per plant
P. altinis	*Passiflora*	13c	5c	0
P. biflora	*Decaloba*	13.5c	4c	0
P. caerulea	*Passiflora*	32b	25b	5b
P. citrina	*Decaloba*	12c	4c	0
P. edulis	*Passiflora*	130a	108a	30a
P. incarnata	*Passiflora*	45b	28b	10b
P. suberosa	*Decaloba*	11c	4c	0
P. yukatanenesis	*Decaloba*	13.3	4c	0

According to Hartmann *et al*. [10] a high percent grafting success does not necessarily mean compatibility as incompatibility can manifest itself later. This was the case in the present study since scions grafted on rootstalks belonging in the decaloba subgenera had only two leaves ten weeks and 5 cm growth one year after grafting. Lack of growth and inability to fruit indicated stunting a common sign of incompatibility. Self grafts had the most rapid

growth rates while *P. caerulea* and *P. incarnate* showed moderate growth and yield. This finding agreed with Gur *et al*., [9]'s observation that some rootstalks had dwarfing effects on scions. In the present study *P. incarnata* and *P. caerulea* rootstocks had a dwarfing effect on *P. edulis* scions.

CONCLUSION

Results from the present study indicated that by establishing the cyanohydrin glycoside type and content in the rootstalk and foliage within *Passiflora* species a rapid rootstock screening technique of graft compatibility can be developed.

Passiflora incarnata and *P. caerulea* need to be screened further for *Fusarium* wilt resistance since they showed potential of becoming alternative rootstalks for *P. edulis*.

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