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Time-dependent Coagulation of Young Shoot Homogenates of Citrus[1]

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Abstract. **Young shoot homogenates from some noncoagulating taxa coagulated either within 5 or 30 minutes depending on the taxon; others did not coagulate even after 30 minutes. Such time-dependent coagulation behavior or its absence is taxon-specific and appears to have uses as an additional marker in taxonomic and genetic studies in *Citrus* and to identify zygotic and maternal seedlings from crosses of 2 polyembryonic cultivars if the parents have the contrasting traits. Genetic data obtained from two F_1 populations, an F_2 population, and a presumed back-cross indicate that the character may be controlled either by 1 or 2 gene pairs.**

Young shoot tissues from *Citrus* taxa either turn into a thick paste or remain thin and watery upon homogenization. The behavior shows taxon-specific distribution and is reproducible. *Citrus* taxa can be classified into 2 phenotypes, coagulating and noncoagulating, on this basis (1). Genetic studies have indicated that noncoagulation is dominant to coagulation and under single gene control (2). The mechanism by which coagulation reaction takes place is not understood.

Tissue homogenates were poured on blotting paper as soon as homogenization was completed (45 to 60 sec) and scored for coagulation or its absence in initial work by Esen and Geraci (1). Recently, it was discovered that homogenates from some noncoagulating taxa coagulated if they stood for some time before pouring. All the taxa which were classified as noncoagulating in the earlier study (1) were consequently re-evaluated in the spring of 1975. Two F_1 populations, an F_2 population, and a presumed backcross were also studied to elucidate the mode of inheritance of the character.

Materials and Methods

A total of 163 taxa were studied, 150 of which belonged to *Citrus* and 13 to related genera. Each taxon is identified with a CRC (Citrus Research Center) accession number. Some taxa had only 1 representative available for study while others, commercially important ones in particular, were represented by numerous cultivars. An F_2 population of 76 individuals from selfing of an interspecific hybrid, 'Acidless' pummelo (*C. grandis* [L.] Osbeck) × 'Kinnow' (*C. reticulata* Blanco), was used for genetic analyses. Progenies from a presumed backcross, 'Clementine' (*C. reticulata*) × ('Acidless' pummelo × 'Frua') [*C. grandis* × *C. reticulata*], and two F_1 populations, 'Sukega' (*C. paradisi* Macf.?) × 4*x* 'Paperrind' (*C. sinensis* [L.] Osbeck)

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Table 1. Segregation for coagulation and noncoagulation in progenies from certain Citrus crosses.

Cross	No. progenies Coagulated by		Noncoagulating by			
	45-60 sec	5 min	30 min	30 min	χ^2	p[z]
Clementine x (Acidless pummelo x Frua)	25	0	0	53	10.05	1%
Sukega x 4*x* Paperrind	40	7	2	109	22.78	1%
Sukega x 4*x* King	35	0	1	35	---	--
Acidless pummelo x Kinnow selfed	14	29	17	16	NA	NA

[z]For an expected 1:1 ratio.

and 'Sukega' x 4*x* 'King' (*C. reticulata*), were also screened (Table 1).

Homogenates of tissue from the terminal 1-3 cm portion of growing shoots were prepared as described by Esen and Geraci (1). Samples of 1 g tissue was homogenized for 45 to 60 sec in 3 ml 50 mM phosphate buffer (pH 7.2) which contained 10 mM K-metabisulfite to inhibit enzymatic browning (3). The slurry was poured on white blotting paper immediately and 5 min and 30 min later, and scored for coagulation or its absence. Thus, 3 successive spots were produced, representing 45 to 60 sec, 5 min and 30 min pours, respectively, from each sample. The spots produced 2 concentric rings (Fig. 1), the inner one from the solid phase and the outer one from diffusion of the liquid phase. Formation of a sharp boundary between the outer and inner concentric rings, and the paste-like condition of the homogenate at the time of pouring were used as criteria for coagulation, the opposite of these conditions being considered as noncoagulation.

Tissue from a noncoagulating taxon inhibits coagulation of tissue from a coagulating taxon when they are homogenized together (1), hence equal amounts of tissue from the 2 types were ground together to ascertain whether such inhibition was also time-dependent.

Cultivars of *C. paradisi* were retested in the Fall of 1976 to investigate if there were seasonal differences in the coagulation behavior of a given cultivar.

A population of progeny trees that presumably resulted from selfing 'Duncan' grapefruit was screened in order to investigate whether coagulation behavior of homogenates could be used as a marker to distinguish nucellar and zygotic progeny from a polyembryonic cultivar. These trees had been identified earlier as nucellar or zygotic on the basis of seedling morphological characters by Walter Reuther.

Results and Discussion

All taxa studied were noncoagulating when homogenates were poured immediately, confirming previous results (1). However, 3 classes were distinguishable when homogenates were allowed to stand for 5 and 30 min, respectively, and then poured: 1) taxa that showed coagulation when poured at 5 min, 2) taxa that showed coagulation at 30 min, and 3) taxa that showed no coagulation after more than 30 min (Table 2). Phenotypes of several taxa, such as *C. volkameriana* and 2 accessions of 'Kulu' lemon, *C. limon,* could not be judged with certainty and they were designated as "intermediate." Time intervals selected, 5 min and 30 min, for the tests were arbitrary and other time intervals might be utilized to distinguish additional classes.

The basic mechanism appears to be the same whether coagulation occurs almost instantaneously or later. There is the formation of an insoluble product from reactants which are likely compartmentalized in the living cell but come in contact with one another when this compartmentalization is disrupted upon homogenization. Esen and Geraci (1) showed that noncoagulating taxa contain a substance(s) which inhibits coagulation of homogenates from coagulating taxa. This inhibition is of short duration. Grinding equal amounts of tissue from a coagulating taxon and a noncoagulating taxon together or grinding tissue from a coagulating taxon in the supernatant obtained by centrifugation of homogenates from noncoagulating taxa inhibits coagulation of tissue from coagulating taxa up to several minutes. Tissues or supernatant from taxa not coagulating within 45 to 60 sec but coagulating by 5 min also inhibits coagulation of homogenates from coagulating taxa for several minutes. Thus, it appears that time-dependent coagulation is related to the concentration of an inhibitor or of reactants of the coagulation reaction. It is proposed that a taxon coagulating within 45 to 60 sec contains reactants but lacks the inhibitor, while one coagulating after 5 or 30 min contains the inhibitor and reactants, the relative concentrations of which determine how sooner or later the coagulation takes place. Those not coagulating after 30 min either have a higher concentration of the inhibitor or are devoid of reactants or both. Four taxa not coagulated at 30 min were tested up to 24 hr; 3, 'Hawaiian' sour orange, 'Royal' and 'Triumph' grapefruit, were noncoagulated and 'Imperial' grapefruit coagulated at the end of 24 hr.

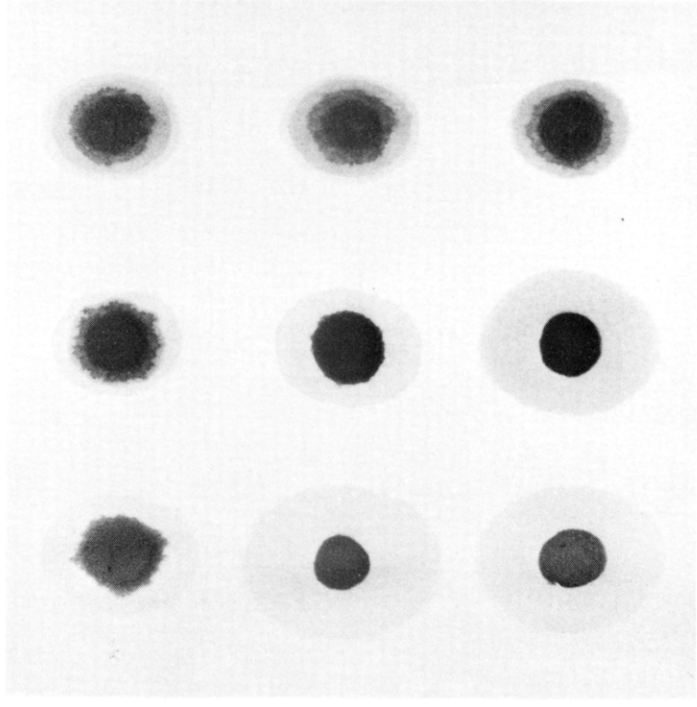

Fig. 1. Fall coagulation tests at 1, 5, 30 min, left to right. Top row, noncoagulating *C. aurantium* cv. Sicilian. Middle row, *C. paradisi* cv. Marsh coagulating at 30 min. Bottom row, *C. grandis* cv. Kao Panne coagulating at 5 min.

Table 2. Coagulation reaction of certain citrus accessions.

Genus and species[z]	Accession name	CRC accession no.
	Coagulation > 1 to 5 min	
C. grandis	Acidless	2240
	African	2346
	Chandler	3224
	Deep Red	2347
	Hunan	1225
	Kao Phuang	2352
	Kao Run Tia	2350
	Nakon Chaisi	2353
	Pink	2244
	Red Fleshed	40
	Roeding's	1208
	Siamese	1220
	Sweet	3067
	Sunshine	2236
	Thong Dei	2589
	Yorba Linda	1183
	Unnamed	2486
C. hassaku[y]		3258
C. webberii Wester		1455
	Coagulation > 5 to 30 min	
C. grandis	Hawaiian	454
	Karn Lau	2341
	Pan Dan	2752
	Pin Shan Kong	2348
	Red	2245
	Tau	2583
	Kao Panne	2356
	Fleming's	578
	Unnamed	1226
	Unnamed	3230
	Unnamed	3236
	Unnamed	950
C. paradisi	Alonzo	3636
	Clason	2566
	Davis	349
	Duncan	3379
	Foster	799
	Hall's Silver	256
	Hamilton	3557
	Howell	320
	Hudson	3638
	Little River	2337
	Marsh (seedless)	1718
	Marsh (seeded)	— — —
	McCarty	265
	Nicholson	3398
	Red Blush	2850-A
C. celibica var. *southwickii* (Wester) Swing.		2453
C. hiroshimana[y]		3275
C. otachibana[y]		3470
C. sinograndis[y]		3148
Eremocitrus glauca (Lindl.) Swing.		3463
	Noncoagulating	
C. medica L.	Citron of commerce	3518
	Corsican	3521
	Diamante	3522
	Dulcia	3654
	Hiawassie	3527
	Indian	3528
	Italian	3530
	Mexican	3531
	Odorato	3655
	Papuan	3532
	Philippine	3533
	South coast	3546
	Sicily	3534
	Spadifora	3535
	Yemen	3536
C. limon (L.) Burm. f.[x]	Cuban (Shaddock)	1462
	Everbearing	3153
	Interdonato	3593
	Khoubs-el-arsa	2489
	Limau mata susu	3755
	Ponderosa	294
	Wild	3300
	Zanzibar	3197
	Unnamed	3047
C. aurantifolia[w] (Christm.) Swing.	Limoui chiri	3263
	Mithi	3051
	Palestine	1482
	Sweet	920
C. sinensis	Orogold	693
C. reticulata	Murcott	3240
C. aurantium	Algerian	2582
	Argentina	2803
	Beladi	2447
	Dummett	2549
	Egyptian	2538
	Granitos	2715
	Hawaiian	2898
	Indian	3089
	Iran	3091
	Laranja de terra	2374
	Merritt's Island	2548
	Oklawaha	2859
	Orlando	1588
	Paraquay	660
	Paradeniya	2887
	Rehoboth	2440
	Rhodesian	2444
	Seville	2441
	Scilian	3131
	Standard	2372
	Stow #20	2192
	Tel Aviv	2435
	Town's Cuban	2858
	Tunisian	2443
	Variegated	622
	var. *salicifolia*	3289
	Chinotto	2375-A
C. paradisi	Imperial	596
	Royal	248
	Triumph	297
C. grandis[x]	Philippine	2343
	Red Aranyan	2608
	Sour	3066
	Unnamed	2558
	Unnamed	2340
C. indica Tan.		3163
C. macropetera Montr.		432
C. hystrix DC		3352
C. ichangensis Swing.		2431
C. micrantha var. *microcarpa* Wester		3605
C. amblycarpa Ochse		2485
C. assamensis Dutta and Bhatt.		3173
C. funadoka[y]		3274
C. glaberrima[y]		3256
C. hanaju[y]		3231
C. junos Sieb.		1216
C. intermedia[y]		3474
C. iyo[y]		3255
C. mediaglobosa[y]		3575
C. megaloxicarpa Lust.		3241
C. miaray Wester		3574
C. mitis Blanco		2592
C. natsudaidai Hay		3235
C. neoarurantium[y]		3468
C. obovoidea[y]		3465

C. pennivesiculata Tan.		2434
C. pseudograndis[y]		3266
C. rokugatsu[y]		3473
C. sudachi[y]		3471
C. taiwanica Tan. and Shim.		2588
C. tamurana[y]		3092
C. tengu[y]		3464
Clymenia polyandra (Tan.) Swing.		3284
Fortunella japonica (Thunb.) Swing.		3237
F. margarita (Lour.) Swing.		3544
F. obovata Tan.		3475
Poncirus trifoliata (L.) Raf.	Argentina	3206
	English large	3548
	Flying dragon	3330
	Pomeroy	1717
	Roubidoux	838
	Town G	3207
	Webber-Fawcett	2552
Misc. hybrids		
C. ichangensis × ?	Ichang (lemon)	2354
(*C. aurantifolia* × *F. japonica*) × *F. hindsii*	Procimequat	3295

[z]Accessions have been assigned to the groups used by Ford (4) where identity was possible.

[y]Species names assigned by T. Tanaka (5) for taxa that he considered distinct but not equivalent to botanical species. All of these species have the suffix, "Hort. ex. Tan."

[x]All of these cultivars are atypical for the species.

[w]These 4 accessions are non-acid limes.

Time-dependent coagulation of young shoot homogenates from some of the taxa previously classified as "noncoagulating" (1) appears to have uses as a marker in citrus taxonomy, genetics and breeding. Such uses have potential especially within *C. grandis* and *C. paradisi* where it is possible to distinguish cultivars that coagulate within 5 or 30 min from those that do not coagulate. In fact, 4 grapefruit and 8 pummelo cultivars that did not coagulate within 30 min were morphologically different from typical grapefruit and pummelos, respectively. Typical pummelo cultivars are distinguishable from those of grapefruit because the former tend to coagulate faster. However, there are seasonal differences in the coagulation behavior of taxa. Some grapefruit cultivars that did not coagulate within 5 min in the spring of 1975 did coagulate within 5 min in the fall of 1976. However, cultivars that did not coagulate within 30 min in 1975 behaved the same in 1976.

Time-dependent coagulation may be useful in the identification of zygotic seedlings from nucellar ones in crosses between noncoagulating taxa and time-dependent coagulating taxa, particularly when the taxa are morphologically similar. Progenies from this type of cross were not available but the results from the selfed 'Duncan' progeny are informative. Nucellar seedlings of 'Duncan' should coagulate within 30 min. Two of 35 trees identified as nucellar on the basis of morphology did not coagulate within 30 min, which suggested that they were of zygotic origin. Many of the zygotic progeny from selfed 'Duncan' would be expected to be like their parent in regard to coagulation. However 6 of the trees identified as zygotic on the basis of seedling morphological characters did not coagulate within 30 min. These results indicate that in appropriate crosses time-dependent coagulation vs. noncoagulation would assist in the identification of zygotic seedlings at an early stage.

Segregation data for coagulation are summarized in Table 1. 'Clementine', 'Paperrind', 'King', 'Frua', and 'Kinnow' had homogenates coagulating within 45 to 60 sec; thus they were phenotypically coagulating. 'Acidless' pummelo was noncoagulating within 45 to 60 sec but coagulating by 5 min. The F_1 hybrid, 'Acidless' pummelo × 'Kinnow' which produced the F_2 population of 76 individuals, was phenotypically like 'Acidless' pummelo. Another F_1 hybrid, 'Acidless' pummelo × 'Frua', did not coagulate within 30 min. Likewise, 'Sukega' did not coagulate within 30 min.

Esen et al. (2) concluded that noncoagulation was dominant to coagulation and the character was under single locus control. This conclusion was based on scoring of progenies from crosses between coagulating and noncoagulating taxa immediately after homogenization was completed. The present results, based upon scoring after 5 and 30 min, suggest that the mode of inheritance is more complex than initially proposed. The crosses, 'Clementine' × ('Acidless' × 'Frua'), 'Sukega' × 4*x* 'Paperrind' and 'Sukega' × 4*x* 'King', may be treated as test-crosses because each cross produced coagulating and noncoagulating progeny, assuming that the noncoagulating trait is dominant and under single locus control. However, 2 of the crosses produced more than twice as many noncoagulating progeny as coagulating, and the differences between an expected ratio of 1:1 and the observed ratios are highly significant (Table 1). Yet, with the cross 'Sukega' × 4*x* 'King' there is almost a perfect correspondence between the expected (1:1) and observed ratios.

Genetic analysis of F_2 progeny seems to support the dominance of noncoagulation to coagulation when the analysis is based upon the phenotypic classes recognized at 45 to 60 sec. This conclusion apparently is not valid however, when the 76 individuals are scored at 5 and 30 min. The F_2 data support the dominance of noncoagulation to coagulation ($\chi^2 = 1.75$; P = 20%) or its opposite ($\chi^2 = 0.63$; P = 50%) as well as semi-dominance of one to the other ($\chi^2 = 3.46$; P = 20%) if one assumes that the 14 individuals whose homogenate appeared to be coagulating by 45 to 60 sec and the 16 whose homogenate was noncoagulating at 30 min were homozygotes and the other 46 were heterozygotes (Table 1).

A digenic mode of inheritance, 1 gene controlling the presence or absence of reactant(s) producing coagulation reaction, the other controlling the presence or absence of the inhibitor was considered in addition to complete dominance and semidominance. The data did not fit any digenic models whether one assumed complete or incomplete dominance for each locus or different modes of epistatic interactions between two loci. Thus, the genetic basis of coagulation or noncoagulation is presently not fully resolved.

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