Polyphenol Oxidase-Catalyzed Browning of Young Shoot Extracts of Citrus Taxa[1]

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Abstract. Based on the occurrence or absence of browning in young shoot extracts, Citrus taxa can be classified into 2 phenotypes: browning and nonbrowning. Browning results from the enzymatic oxidation of phenolic substrate present in the browning taxa. The enzyme responsible for browning is polyphenol oxidase (*ortho*-diphenol oxidase, EC 1.10.3.1). Nonbrowning taxa lack the substrate(s) and have little or no polyphenol oxidase activity. Browning appears to be a dominant trait. It should be useful as a genetic marker and a taxonomic criterion.

Browning of plant tissue extracts resulting from enzymatic oxidation of phenolic substrates is commonly observed. The enzymes catalyzing the oxidations are polyphenol oxidases (*o*-diphenol oxidase, EC 1.10.3.1). The initial product of oxidation of phenolic compounds is usually *o*-quinones. Being highly unstable, they undergo polymerization to yield brown pigments of high molecular wt and react with amino acids, peptides, and proteins (7, 10, 11, 15, 16).

During a search for marker proteins in *Citrus* spp. using extracts from young shoots, we observed that aqueous extracts from certain taxa rapidly turned brown whereas those of others retained their original green-yellow color. This observation prompted a survey of *Citrus* and some related genera with respect to extract browning.

Materials and Methods

We tested 34 taxa in the genus *Citrus*, 1 in *Poncirus,* 1 in *Fortunella,* 1 in *Microcitrus,* 1 in *Severinia,* and 14 hybrids of known or presumed parentage (Table 1). Use of botanical names follows the classification scheme of Swingle except for *C. macrophylla, C. webberii* var. *montana, F. obovata,* and *M. virgata.* Extracts were prepared from the terminal 1 to 3-cm portion of growing shoots. They were collected in polyethylene bags, immediately placed in an ice chest, and used for extractions within 3 to 4 hr after collection. Unless otherwise indicated, all operations were carried out at 0-4°C.

Extraction. The tissue was homogenized in 0.05-M phosphate buffer at pH 7.2; the ratio of fresh wt to buffer vol was 1:3. The brei was centrifuged for 20 min at 27,000 *g*. The supernatant was decanted into a beaker and left standing. About 24 hr later it was recentrifuged and the color of the supernatant fluid was scored using Munsel color charts (8). Color names were designated according to Kelly and Judd (3). For some taxa, an additional extraction was made. The supernatant in this case was dialyzed against 3 changes of deionized distilled water to find out if the pigment was dialyzable. A second batch of tissue from each taxon was ground and the homogenate was poured onto white desk blotting paper and the spots so formed were scored for browning.

Test for substrate and enzyme. Preliminary tests showed that browning occurred in boiled extracts (free of enzyme) of 'Willow Leaf' mandarin when its own crude extract was added as an enzyme source. The crude extract of this cultivar turned brown faster than others, suggesting that it was rich in enzyme and/or substrate. It was selected as the crude enzyme and substrate source to test other taxa for enzyme and substrate. For this test, immediately after centrifugation, 4 ml of supernatant was dipped in boiling water in a test tube, gently swirled for 1 min, and then filtered through Whatman #1 paper (this treatment resulted in total loss of polyphenol oxidase activity). Two ml of the filtrate was pipetted into a test tube to which was added 0.2 ml of crude extract from each taxon. These reciprocal "enzyme"-"substrate" mixtures between 'Willow Leaf' and other taxa were left at room temperature for about 3 days and scored for browning.

Spectrophotometric assay of polyphenol oxidase activity. The assay was made according to the method of Stafford and Galston (14). Activity was measured by following the appearance of a red-brown product at 475 nm in a 3-ml vol containing 2.8 ml 14 mM DL3,4-dihydroxyphenylalanine (DOPA) in 0.05-M phosphate buffer at pH 6.0 and 0.2 ml of crude extract. The blank contained all the components except crude extract (enzyme). Polyphenol oxidase activity (abbreviated hereafter as PPO) was expressed as increase in absorbance per min per g fresh wt.

Inhibition of browning. We tested a phenol adsorbant, some antioxidants and PPO inhibitors (1, 7) to prevent browning. Treatments included extraction with 2% insoluble polyvinyl pyrrolidone (PVP), 20 mM L-cystein-HCl, 10 mM potassium metabisulfite, 5mM potassium metabisulfite + 1% PVP, 0.5 mM mercaptobenzothiazole, 20 mM diethyldithiocarbamate (DIECA), and plain buffer (as control). One-half of each extract from the above treatments was dialyzed and the other half was left standing. Colors of dialyzed and undialyzed extracts were compared with those of their respective controls.

Results

Extract colors before browning. Color of extracts before browning varied from pale greenish yellow to light orange yellow. Exceptional extracts were those from *Severinia buxifolia* and from *Microcitrus* 'Sydney Hybrid' (hereafter called *M. virgata*) which resulted from the cross *M. australis* (Planch.) X *M. australasica* (F. Muell.) These extracts were purple-black due to heavy anthocyanin pigmentation in young shoots. A rather slight purplish tinge was also visible in extracts from *C. limon* ('Eureka' and 'Lisbon' in particular), *C. hystrix* and *C. macroptera* as a result of anthocyanin pigmentation.

Change in extract color. Taxa fell into 2 distinct phenotypes based on the development or non-development of browning when extracts were left standing. 1) Nonbrowning: extract color showed no visible browning even after 2 weeks. Such taxa are designated with NB in Table 1. 2) Browning: extracts, starting on the surface, gradually turned brown within about 24 hr. Browning was so rapid in certain taxa, especially in cultivars of *C. reticulata,* that it was visible on the surface of supernatant fluids after 20 min of centrifugation. This necessitated handling 1 or 2 samples at a time, in order to reduce the time lapse between homogenization and centrifugation. Browning was slowest and least in intensity in cultivars of *C. paradisi.* Extracts developed browning much more rapidly during dialysis than they did when left standing in beakers or test tubes. The intensity of the final color among the browning phenotypes varied considerably. For example, color was deep orange in *C.*

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paradisi which was easily distinguishable from the nonbrowning and other browning taxa. In others, color varied from strong brown to dark, reddish or blackish brown. Browning or its absence was in general species-specific with the exception of *C. aurantifolia* (Fig. 1-7, 8) and *C. limon* (Table 1) (Fig. 1-5, 6, 9, 10) in which both phenotypes were found. Figure 1-1 to 1-23 show some representative extracts from browning and nonbrowning phenotypes.

Browning began to appear in tissue homogenates poured on the blotting paper within 1 to 10 min, reaching its maximum intensity within an hour or so. Cultivars of *C. paradisi* failed to develop browning on the paper and were indistinguishable from nonbrowning taxa.

A small amount of the brown pigment was dialyzable and this was evident by slight coloring of the first change of water. Exhaustive dialysis (72 hr, 6 changes of water) showed that the rest of the pigment remained in solution in the dialysis bag.

Browning-substrate-enzyme relationships. Results of reciprocal mixings of boiled extracts (substrate) and crude extracts (enzyme) showed that all the browning phenotypes contained the substrate(s) oxidized by the enzyme from 'Willow Leaf' and turned brown (Table 1, Fig. 2-2b, 5b, 8b-12b). Such taxa were considered to have given a positive test for the substrate. Although intensity of color was on the average comparable to that of crude extracts left standing, browning took place at a slow rate. No browning was detectable in boiled extracts from nonbrowning phenotypes when mixed with the enzyme from 'Willow Leaf'. They are considered to lack the substrate(s) (Table 1, Fig. 2-1b, 3b, 4b, 6b, 7b, 13b, 14b). In the case of *Severinia buxifolia* and *Microcitrus virgata* (Fig. 2-15b, 16b) results were inconclusive because the boiled extract of these taxa was initially purplish-black.

When the crude extract of each taxon was mixed with the boiled extract of 'Willow Leaf' all the browning phenotypes with the exception of *C. paradisi* (Fig. 2-2a) catalyzed the browning of the boiled 'Willow Leaf' extract, thus yielding a positive test for the enzyme (Table 1, Fig. 2-5a, 8a-12a). The rate and final intensity of browning was closely related to the total PPO activity of each taxa. The crude extract of the non-browning phenotypes as well as that of *Severinia, Microcitrus,* and *C. paradisi* failed to produce any detectable browning in the boiled extract from 'Willow Leaf' and thus appeared to lack the enzyme (Table 1, Fig. 2-1a-4a, 6a, 7a, 13a-16a).

Polyphenol oxidase activity of crude extracts. Spectrophotometric assays using DOPA as substrate indicated that total activity varied from 0.00 to 0.07 (absorbance/min/g.f.wt.) in the nonbrowning taxa and from 0.05 to 4.18 in the browning ones (Table 1). The lowest activity among the browning taxa was measured in cultivars of *C. paradisi*; the highest, in those of *C. reticulata* and *C. tachibana* (Table 1). There were wide differences both between and within species. Change in absorbancy was always linear. Because crude extracts, those of the browning taxa, *Severinia* and *Microcitrus*

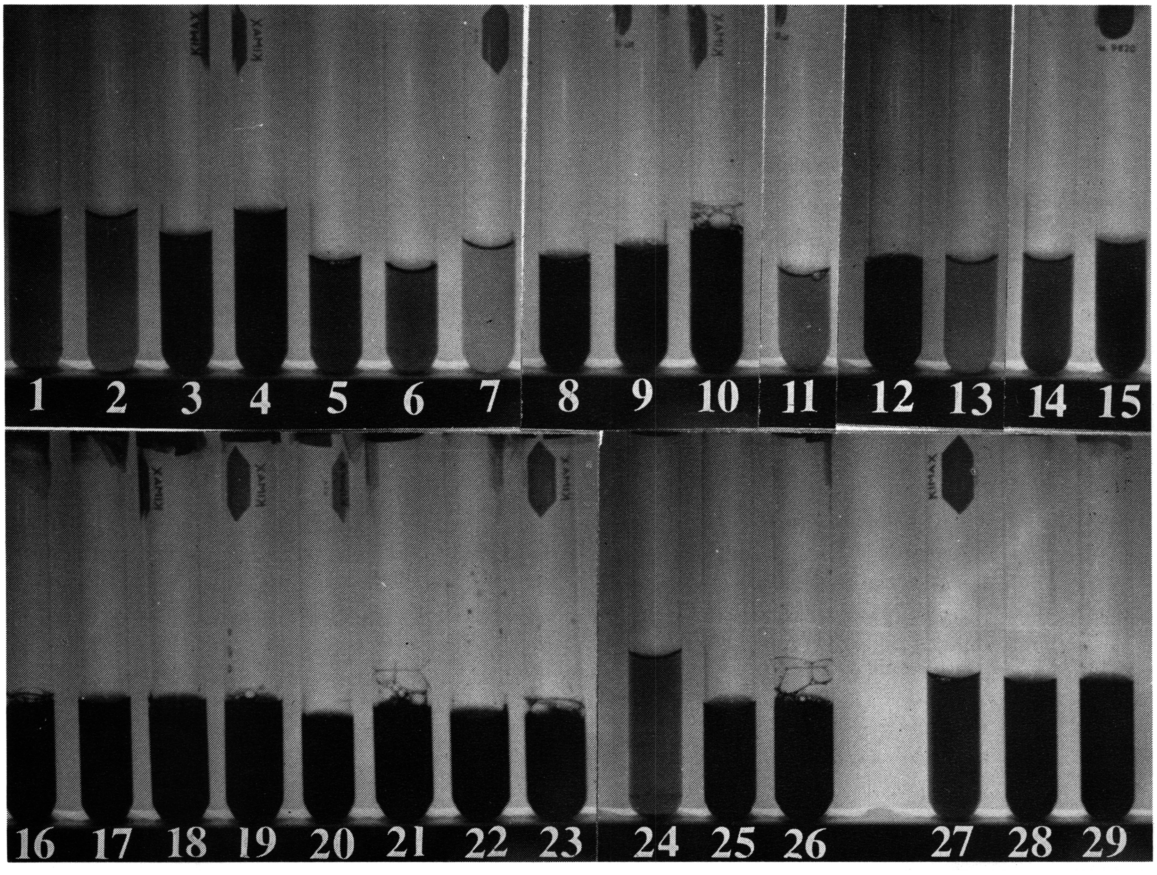

Fig. 1. Crude extracts from some representatives of the browning and nonbrowning *Citrus* and related taxa. 1-2. *C. grandis* (1. 'Acidless', 2. 'Deep Red' pummelo). 3-4. *C. paradisi* (3. 'Imperial', 4. 'Foster Pink' grapefruit). 5-6, 9-10. *C. limon* (5. 'Lisbon', 6. 'Eureka', 9. 'Florida Rough', 10. 'Indian Rough' lemon). 7-8. *C. aurantifolia* (7. 'West Indian', 8. 'Palestine Sweet' lime). 11. *C. latipes.* 12. *C. tachibana.* 13. *C. macrophylla.* 14. *Poncirus trifoliata* ('Rubidoux' trifoliate). 15. *Fortunella obovata* ('Fukushu' kumquat). 16-17. *C. sinensis* (16. 'Washington Navel', 17. 'Valencia' orange). 18-19. *C. aurantium* (18. 'Seville', 19. Sour orange). 20-23. *C. reticulata* (20. 'Satsuma', 21. 'Kinnow', 22. 'Ponkan', 23. 'Cleopatra' mandarin). 24-29. Parent-hybrid relations (24. 'Acidless', 25. 'Acidless' X 'Kinnow', 26. 'Kinnow', 27. 'Imperial', 28. 'Imperial X Willow Leaf' ('Pearl' tangelo), 29. 'Willow Leaf'). The reader should note that black and white photographs only illustrate differences between browning and nonbrowning taxa, not within. Also, if the extract from a nonbrowning phenotype was cloudy or had more of a green tinge, which was the case with Fig. 1-15, it appeared almost black in black and white photographs.

in particular, had some absorbancy at 475 nm, addition of the extract to the substrate gave initial absorbancy readings from 0.02 to 0.15. This initial reading was 0.52 in *Microcitrus virgata*. A surprising observation was the linear decrease in absorbancy

Table 1. Extract color browning and polyphenol oxidase activity in *Citrus* and related genera.

CRC accession number	Taxa	Extract color[z]	Substrate[y]	Enzyme[y]	PPO activity[x]
	C. medica (L.)				
3521	Corsican citron	NB	–	–	0.00
3531	Mexican citron	NB	–	–	0.04
	C. limon (L.) Burm. f.				
3176	Lisbon lemon	NB	–	–	0.07
3013	Eureka lemon	NB	–	–	....
400	Rough lemon (Florida)	B	+	+	0.60
3061	Rough lemon (Australia)	B	+	+	0.32
3060	Rough lemon (India)	B	+	+	....
3093	Sweet lemon	B	+	+	0.42
	C. aurantifolia (Christm.) Swing				
1813	West Indian lime	NB	–	–	0.02
1482	Palestine Sweet lime	B	+	+	....
	C. grandis (L.) Osbeck				
2240	Acidless pummelo	NB	–	–	0.00
3324	Chandler Pink pummelo	NB	–	–	....
1220	Siamese pummelo	NB	–	–	....
2347	Deep Red pummelo	NB	–	–	0.00
	C. paradisi Macf.				
799	Foster Pink grapefruit	B	+	–	....
3379	Duncan grapefruit	B	+	–	0.04
596	Imperial grapefruit	B	+	–	0.05
1718	Marsh Seedless grapefruit	B	+	–	0.04
	C. sinesis (L.) Osbeck				
3270	Washington Navel orange	B	+	+	0.46
3339	Werley Valencia orange	B	+	+	0.71
	C. aurantium L.				
1589	Seville Sour orange	B	+	+	1.39
2372	Sour orange	B	+	+	....
	C. reticulata Blanco				
270	Cleopatra mandarin	B	+	+	....
2332	Ponkan mandarin	B	+	+	....
2567	Satsuma mandarin	B	+	+	1.88
3026	Dancy mandarin	B	+	+	2.70
595	Willow Leaf mandarin	B	+	+	4.18
279	Clementine mandarin	B	+	+	1.76
2455	*C. macrophylla* Wester	NB	–	–	0.00
3150	*C. tachibana* (Mak.) Tan.	B	+	+	3.19
767	*C. webberii* var. *montana* Wester	B	+	+	0.45
3352	*C. hystrix* DC.	NB	–	–	0.00
432	*C. macroptera* Montr.	NB	–	–	0.00
3090	*C. latipes* (Swing.) Tan.	NB	–	–	0.03
	Poncirus trifoliata (L.) Raf.				
838	Rubidoux trifoliata	NB	–	–	0.00
	Fortunella obovata Tan.				
3475	Fukushu kumquat	NB	–	–	0.00
1485	*Microcitrus virgata* (Sydney hybrid)	NB?	–?	–	– 0.20[w]
1492	*Severinia buxifolia* (Poir.) Tenore	NB?	–?	–	– 0.07[w]
	Hybrids				
	C. reticulata X *C.* sinensis?				
303	King tangor	B	+	+	....
2417	Temple tangor	B	+	+	....
	C. paradisi X *C. reticulata*				
3340	Minneola tangelo (Duncan X Dancy)	B	+	+	1.99
2790	Orlando tangelo (Duncan X Dancy)	B	+	+	1.17
2873	Clement tangelo (Duncan X Clementine)	B	+	+	0.41
2849	Pearl tangelo (Willow Leaf X Imperial)	B	+	+	0.45
2871	Unnamed tangelo (Satsuma X Imperial)	NB	–	–	0.03
	C. paradisi X *C. sinensis*				
	Sukega orangelo? (Imperial X Blood orange)[v]	B	+	–	....

Table 1 (Continued)

650	Unnamed	B	+	+	....
	(C. reticulata X *C. sinensis?)* X *(C. reticulata*				
3021	Kinnow mandarin (King X Willow Leaf)	B	+	+	2.20
3020	Wilking mardarin (King X Willow Leaf)	B	+	+	....
	C. grandis X [*C. reticulata* X *C.* sinensis?) X *C. reticulata*]				
	Unnamed (Acidless X Kinnow)[v]	B	+	+	0.55
	Unnamed (Acidless X Wilking)[v]	B	+	+	....
	Poncirus trifoliata X?				
3550	Unnamed	B	+	+	0.60

[z](B) browning, (NB) nonbrowning

[y]Based on qualitative tests. + indicates presence, – absence.

[x]Activity expressed as absorbance/min/g.f.wt.

[w]Decrease in absorbance may not be associated with PPO activity.

[v]No assigned accession number.

following addition of extracts from *S. buxifolia* (-0.07) and *M. virgata* (-0.20) to the substrate (Table 1). Assays had to be made immediately after extraction because activity decreased as browning progressed. This was verified by following the loss of activity in crude extract of 'Willow Leaf'. Activity was 4.18 soon after extraction, 0.72 and 0.22 after 24 and 48 hr, respectively. Dialysis of extract resulted in total loss of activity.

The enzyme showed no affinity for chlorogenic acid and failed to oxidize it. No other known PPO substrates were tested.

Effect of inhibitors. Insoluble PVP and mercaptobenzothiozole did not prevent browning. In contrast, potassium metabisulfite, DIECA, and cystein totally inhibited browning. When extracts were dialyzed, those containing metabisulfite treatment alone or in combination with PVP showed no evidence of color change.

Extract color in hybrids. Hybrids from known or presumed parentage between browning taxa as well as those between browning and nonbrowning taxa were of the browning phenotype (Fig. 1-24 to 1-29). They also gave a positive test for the enzyme and substrate (Table 1). The only exception was an unnamed hybrid between 'Owari Satsuma' and 'Imperial' grapefruit. It was of the nonbrowning phenotype and yielded a negative test for the enzyme and substrate. PPO activity of hybrids indicated no predictable pattern (compare PPO activities of parents and hybrids listed in Table 1). As mentioned before, cultivars of *C. paradisi* behaved like nonbrowning taxa when their extracts were tested on blotting paper. The 2 triploid hybrids (not listed in Table 1) from a cross between 'Acidless' pummelo and tetraploid 'Seedy Marsh' grapefruit behaved like their parents (nonbrowning) in tests on blotting paper. However, 14 hybrids (not listed in Table 1) between these triploids and 'Dancy' were all of the browning phenotype. These results suggest that browning is very likely a dominant trait and dominance is expressed with the production of the substrate(s) oxidized by PPO.

Discussion

Results of this study show that Citrus taxa can be divided

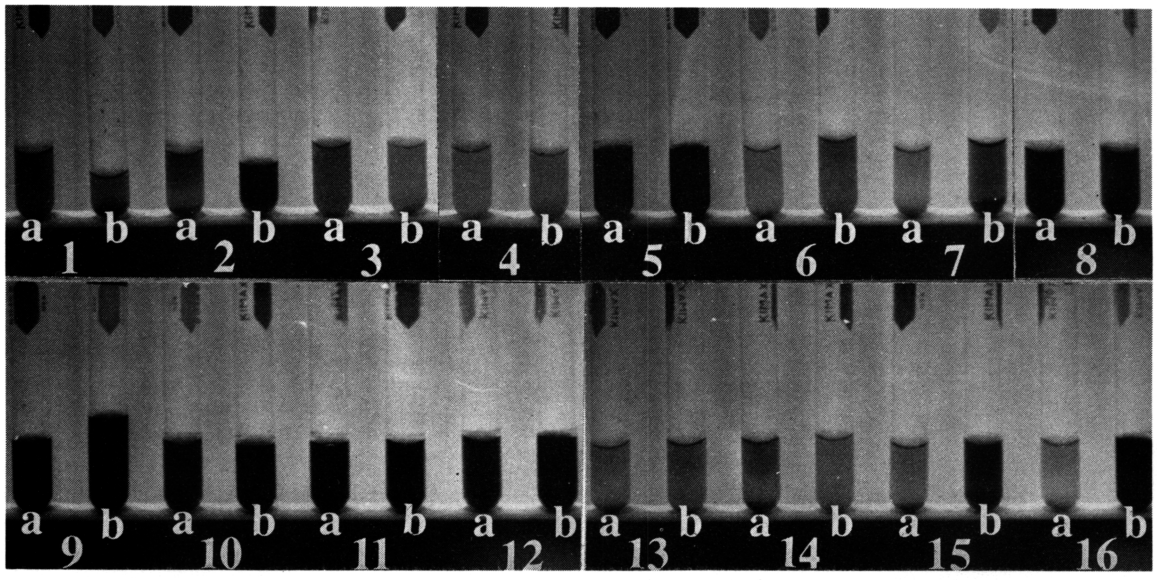

Fig. 2. Color of boiled extracts following reciprocal "enzyme" – 'substrate' mixings between 'Willow Leaf' mandarin and browning and nonbrowning taxa. In all pairs (a) shows color development in boiled 'Willow Leaf' extract mixed with the crude extract from designated taxa (b) boiled extract of designated taxa mixed with crude extract from 'Willow Leaf'. (See details in Materials and Methods). 1. *C. grandis* ('Acidless' pummelo). 2. *C. paradisi* ('Foster Pink' grapefruit) 3. *C. limon* ('Lisbon' lemon). 4. *C. aurantifolia* ('West Indian' lime). 5. *C. tachibana.* 6. *C. macrophylla.* 7. *C. hystrix.* 8. *C. aurantium* ('Seville' sour orange). 9-12. *C. reticulata* (9. 'Satsuma', 10. 'Kinnow', 11. 'Ponkan', 12. 'Cleopatra' mandarin). 13. *Poncirus trifoliata* ('Rubidoux' trifoliate). 14. *Fortunella obovata* ('Fukushu' kumquat). 15. *Microcitrus virgata.* 16. *Severinia buxifolia.*

into 2 distinct phenotypes based on browning or its absence in aqueous extracts of young shoots. The sequence of events leading to this phenomenon is probably similar to that described by Wright et al. (15, 16), Pierpoint (9, 10, 11) and Loomis (6, 7); that is, oxidation of phenolic compounds to quinones followed by their condensation to form polymers of high molecular wt as well as their binding covalently with the $-NH_2$ and $-SH$ groups of proteins. Wright et al. (15, 16) showed that the browning pigments of aged 'Burley' tobacco consisted of 1): the non-dialyzable fraction which was an iron-protein-chlorogenic acid-rutin complex (MW. 20,000-30,000) and 2) the dialyzable fraction which was a protein-chlorogenic acid complex (MW. 4,000). Our observations indicate the presence of similar fractions in *Citrus*. The dialyzable fraction was evident in the slight browning of the first change of water during dialysis. The fraction retained within the dialysis bag most likely had a molecular weight above 10,000 because the dialysis tubing used was designed to permit the passage of compounds having molecular weights below 10,000. Most of the non-dialyzable fraction appears to be protein-bound. When dialyzed extracts were subjected to polyacrylamide gel electrophoresis, the brown pigment formed a sharp brown band and moved as the fastest anodal band. Mobility of this band was identical with that of bromophenol blue tracking dye and it was stained with commassie blue like any other protein bands. When proteins were precipitated by treating the extract with 12.5% tricholoroacetic acid, the precipitate was chocolate color and the supernatant retained only small amounts of pigment which might represent a protein-free polymer of high molecular wt.

The browning did not occur in boiled extracts, if the enzyme was not added; thus autoxidation of the substrate was negligible, if any. The enzyme catalyzing the oxidation of the substrate was PPO as indicated by the following results: first, the rate of browning in a given extract was directly related to total activity measured, using DOPA as substrate; the higher the activity, the more rapidly the browning occurred. Secondly, the enzyme was inhibited by known PPO inhibitors such as DIECA (9) and metabisulfite (1). Wide variation in PPO activity among the browning taxa was perhaps due to their having different isoenzymes of the enzyme with different kinetic properties and concentration. The fact that the nonbrowning taxa had little or no activity could be attributed to the absence of the enzyme in these taxa or to their having forms with little or no activity. Moreover, the data suggest that the presence of enzyme was dependent on the presence of substrate because in all nonbrowning taxa the absence of substrate was accompanied by little or no enzyme activity. However, the situation found in cultivars of *C. paradisi* in which activity was low in the presence of substrate argues against such an "inducible" system.

The rapid browning of extracts during dialysis and of homogenates on blotting paper must have been due to increased availability of O_2. This conclusion is supported by the appearance of browning first on the surface of the extract in beakers or test tubes. The decrease in enzyme concentration was certainly the reason for rather slow rate of browning in boiled extracts because in all cases the ratio of vol of the boiled extract to that of the crude enzyme preparation was 10:1, representing a 10-fold dilution. This dilution effect could also explain the failure of crude extracts from cultivars of *C. paradisi* to oxidize the boiled extract of 'Willow Leaf'. Low enzyme activity probably also was responsible for the lack of browning on blotting paper observed in homogenates of *C. paradisi* cultivars. Because homogenates dried on the paper in an hour or so, homogenates with low activity probably had insufficient time to produce visible browning.

Although we were able to demonstrate that browning phenotypes contain a substrate(s) which was not present in nonbrowning ones, we do not as yet know its identity and structure. It may consist of one or several compounds that could serve as a substrate for PPO. It was observed that the addition of a drop or 2 or 3% H_2O_2 produced browning almost instantly in boiled or crude extracts of the browning taxa. Such browning also occurred in extracts of the nonbrowning taxa but its rate was slow and the color was much lighter than that observed in the browning taxa. The difference between the browning and nonbrowning phenotypes may be that the former have a compound(s) that serves as substrate for PPO and the latter lack it (them). Levings and Stuber (4) reported that maize inbred lines with silk browning contained about 12 chromatographically different *o*-dihydroxyflavones which were not present in inbreds with no silk browning. An analogous situation may very well exist in *Citrus*. It was ascertained that the substrate(s) was of low molecular wt because we could not produce browning of boiled extracts enzymatically after they were dialyzed.

Because phenolic compounds, their oxidation products, and their interactions with proteins interfere with isolation of proteins and organelles, inhibit certain enzymes, and modify properties of proteins, the importance of their removal or the inhibition of PPO has been stressed by various workers (1, 2, 5, 6, 7). Similar problems can be expected in biochemical studies involving *Citrus* in view of extract browning and its association with PPO activity. Browning can be prevented by including cystein, metabisulfite and DIECA in the extraction buffer. However, it is not known if these compounds have any effects on the quantity and/or quality of proteins or enzymes extracted. Nor is it known if they inhibit or modify enzymes other than PPO.

The fact that crosses between the browning and nonbrowning parents produce hybrids of the browning phenotype strongly suggests that browning is a dominant trait. Single locus control of silk browning and its dominance in maize has recently been reported (4). Studies involving an F_2 population in *Citrus* so far indicate a simple mode of inheritance.

Adventive embryony has been one of major factors hampering genetic studies and breeding in *Citrus* because the zygotic progeny of a cross cannot be separated from maternal ones in most cases until fruiting, especially if crosses involve closely related parents. Genetic markers permitting early screening for zygotic individuals among the progeny from polyembryonic pistillate parents are needed. The only available useful marker, trifoliate leaf, resides in another genus, *Poncirus*. Browning of extracts promises to be a useful marker for distinguishing the zygotic progeny at the seedling stage after crosses between nonbrowning polyembryonic pistillate parents and browning staminate parents. Screening of the progeny can be initiated as soon as 0.2 to 0.5 g of surplus young shoot growth is available from seedlings. Porspective staminate parents with the browning phenotype should be tested for heterozygosity by crossing them with a member of the nonbrowning taxa. The occurrence of a non-browning hybrid from a cross between 2 browning parents ('Owari Satsuma' and 'Imperial' grapefruit) (Table 1) indicates that these taxa are heterozygous for browning.

Data on the PPO activity of the browning taxa and of hybrids between the 2 phenotypes were so variable that no inferences could be made about the mode of inheritance of the enzyme activity. Undoubtedly, the enzyme exists in multiple molecular forms each coded by one or more loci and these molecular forms vary in their kinetic properties as well as concentration in different genetic backgrounds. This could account for wide variation in measured activities.

Because extract browning or its absence largely appears to be species-specific, it may be useful as a taxonomic criterion to supplement other criteria used to analyze species relationships. The species-specificity of extract color did not hold in *C. limon* and *C. aurantifolia* in which both phenotypes are found. Within

C. limon the "true lemons" ('Lisbon' and 'Eureka') had nonbrowning extracts. Three "rough lemons" ('Florida', 'Indian', and 'Australian') were of the browning phenotype suggesting that they represent a distinct group. This observation supports the conclusion reached by Scora et al. (13) and Scora and Malik (12). These authors showed, by gas chromatography of leaf essential oils, the existence of 2 subgroups, "true lemons" and "rough lemons", within *C. limon.* The cv. Palestine Sweet lime and West Indian lime (*C. aurantifolia*) differed with respect to their extract colors the former was browning and the latter nonbrowning. That difference suggests the presence of subgroups within *C. aurantifolia.* A more extensive study of *Citrus* and related genera is needed before the utility of extract color as a taxonomic criterion can be fully determined.

Extract color browning also may be useful when the identity of 1 parent of a hybrid is in question. For example, the cv. Sukega was supposed to be from a cross between 'Imperial' grapefruit and a blood orange. Because 'Sukega' lacked orange-like characters it was suspected that it did not originate from the intended cross. Its extract behaves like those of other grapefruit cultivars thus reenforcing the suspicion that it resulted from either accidental selfing or crossing with another grapefruit.

Our data indicate that the presence of browning or nonbrowning phenotypes in *Citrus* may prove useful in breeding work and in genetic and taxonomic studies. More extensive studies are needed for the identification of phenolic substrate (or substrates) responsible for browning and for the purification, characterization, and distribution of the enzyme (PPO) catalyzing the browning reactions.

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