Table 1 *Valine, isoleucine and leucine levels in Glycine* and corn suspension cultures (nmol/g fresh weight)

Cell Strain	Valine	Isoleucine	Leucine	Protein amino acids
Corn	233.5	88.5	103.5	3203
Old Dominion	912.5	647.5	659	11060
G. max A3127	471	462	305 5	7537
G. canescens	687	548.5	470	9821
Earliana	398.5	262.5	350.5	5367.5

the most sensitive, had higher free amino acid levels than the more DPX-F6025 tolerant Earliana cells with the lowest total free amino acid content.

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ISOLATION AND TENTATIVE CHARACTERIZATION OF CERTAIN GENE-CONTROLLED SUBSTANCES IN ANTHOCYANIN BIOSYNTHESIS IN MAIZE

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GENETIC control of anthocyanin biosynthesis in maize has been of great interest since it provides a model system in the study of gene action and gene regulatory mechanisms[1]. About 14 loci with several alleles, a number of modifiers and dominant inhibitor (CI), are known to control the synthesis of anthocyanin and related pigments in the aleurone and other plant tissues[2]. The dominant gene, CI blocks the synthesis whereas recessive intensifier (in), bronze-1 (bz1) and bronze-2 (bz2) control the intensity of anthocyanin, and Pr/pr controls the hydroxylation pattern. Based on inter-tissue complementation, studies with fresh 25-day-old aleurone tissue pairs, two at a time, in all possible combinations, led to the analysis of the following linear gene action sequence: CI-C1-C2-R-(In)-A1-A2-Bz1-Bz2-Cyanidin-3-glucoside[3].

A study of the intermediates and gene-controiled accumulated substances of desirable single and double recessives including inhibitor may reveal the step-wise process of biosynthesis of anthocyanin in maize. Earlier studies with single and double recessive mutant aleurone tissue have led to the elucidation of specific role of the genes in gene action sequence[4–8].

The present paper, mainly deals with the characterization of accumulated substances controlled by dominant CI, double recessive C2 pr, bz1 pr and r-r in aleurone tissue. These stocks were grown in the field under normal irrigation. Dry mature kernels were collected from field-grown plants of CI, r-r, c2 pr and bz1 pr plants. Pericarp was removed by presoaking in water for 10 min. About 1 kg seed was powdered, defatted with petroleum ether (40–60°C) for 24 hr and then extracted with cold methanol. The extract was concentrated under reduced pressure, followed by hydrolysis of glycosides with 7% H_2SO_4 by volume in methanol by refluxing for 5 hr. By repeated addition of water (10 ml) and removal of methanol (10 ml) by rotatory vacuum evaporator, the methanol and sulphuric acid were removed completely. The aglycone so precipitated was washed several times with water

and was used for chromatography. Repeated chromatography on Whatman No. 1 with *n*-BuOH, HOAC, H_2O [*n*-butanol : 27% aqueous acetic acid (1 : 1 V/v)], phenol, water (73 : 27 W/W) and Forestal (glacial acetic acid: conc. HCl : water 30:3:10) as solvent systems were used for purification. Final purification was however achieved by preparative TLC using E to Ac and MeOH.

R_f values of the extracted samples along with authentic samples are presented in table 1.

The compounds isolated from the extract of dominant CI gave positive tests for its acidic nature and for the presence of isolated double bond with chemical tests. Phenolic nature was confirmed by red colour with alcoholic $FeCl_3$. UV data λ_{max}^{MeOH} 204 nM (log *E* 4.3), 215 nm (log *E* 4.45), 244 nm (log *E* 4.0), 296 nm (log *E* 4.1) 235 nm (log *E* 4.42) suggested the characteristics of caffeic acid. The IR spectrum of this compound also showed absorptions similar to those of hydroxy cinnamic acid[9]. On the basis of the data obtained on IR, NMR and mass, the compound isolated from the CI (inhibitor) aleurone tissue extract, was the 3,4 dihydroxy cinnamic acid (caffeic acid). These observations were further confirmed by co-paper chromatography superimposable UV and IR with an authentic sample of caffeic acid.

From the aleurone extract of recessive r-r, one of the isolated compounds gave the red colour with conc. H_2SO_4 showing that it is a chalcone and the pinkish colour with alcoholic $FeCl_3$ gave the indication for the presence of phenolic hydroxyl group. Under UV light the spot showed yellow flouresscence. The UV data[10] λ_{max}^{MeOH} 239 sh (log *E* 4.18), 266 (log *E* 3.99), 319 sh (log *E* 4.20), 379 (log *E* 4.44) suggested the characteristics of chalcone. These observations were further confirmed by co-paper chromatography (table 1) superimposable UV and IR with authentic sample of 2′,4′,3,4-tetra hydroxy chalcone (butein). Thus the isolated compound is 2′,4′,3,4-tetra hydroxy chalcone (butein). Another compound from the same extract gave a positive orange colour for the presence of a flavonoid skeleton with Shinoda test[11], and the dark coloration with alcoholic $FeCl_3$ gave the indication of phenolic hydroxyl group. Under UV light the spot showed vellow flourescence. The UV data[12] λ_{max}^{MeOH} 248 (log *E* 4.30), 262 sh (log *E* 4.14), 307 sh (log *E* 4.15), 319 (log *E* 4.24), 362 (log *E* 4.43) suggested the characteristics of a flavonol. These

Table 1 *R_f values of isolated compounds from anthocyanin mutants along with authentic compounds*

	Solvent systems and R_f values							
	1	2	3	4	5	6	7	8
Isolated compound from CI CI	0.86	—	0.45	0.78	—	—	—	—
Authentic sample Caffeic acid	0.86	—	0.46	0.78	—	—	—	—
Isolated compound from r-r	0.83	0.64	—	—	0.07	0.70	—	—
Authentic compound Butein	0.83	0.65	—	—	0.07	0.70	—	—
Isolated compound from r-r	—	—	—	0.58	0.02	0.56	0.73	—
Authentic compound Fisetin	—	—	—	0.58	0.03	0.56	0.73	—
Isolated compound from c2pr	—	0.82	0.67	0.84	—	—	—	0.93
Authentic compound *p*-coumaric acid	—	0.82	0.66	0.84	—	—	—	0.93
Isolated compound from bz1 pr	0.92	0.88	0.97	0.83	—	—	—	—
Authentic compound Apigenin	0.92	0.88	0.96	0.83	—	—	—	—

1. *n*-butanol: 27% aq. acetic acid (1:1 v/v); 2. m-cresol: acetic acid: water (50:2:48 v/v); 3. phenol: water (73:27 w/w); 4. Glacial acetic acid: conc. HCl: water (30:3:10 v/v); 5. 15 ml of reagent grade glacial acetic acid mixed with 85 ml of water; 6. 3:1:1 solution of reagent grade tertiary butanol: reagent grade glacial; 7. Butanol-acetic acid and 8. *n*-butanol: glacial acetic acid: water (4:1:5 v/v).

observations were further confirmed by co-paper chromatography (table 1) superimposable UV and IR with an authentic sample of 7,3′,4′-tri hydroxy flavonol (fisetin); thus the isolated compound is fisetin.

The isolated compound from the extract of double recessive c2 pr gave a positive test for its acidic nature and for the presence of isolated double bonds with chemical tests. Phenolic nature was confirmed by red colour with alcoholic $FeCl_3$. UV data λ_{max}^{MeOH} 210 nm (log E 3.90), 218 nm (log E 3.8) and 285 nm (log E 4.2) suggested that it is a hydroxy cinnamic acid. It is further confirmed by co-paper chromatography (table 1) superimposable UV and IR with an authentic sample of *p*-hydroxy cinnamic acid[13] (*p*-coumaric acid) that the isolated compound is *p*-coumaric acid.

The aleurone extract of double recessive bz1pr was analyzed by different chemical tests. Positive orange colour for the presence of a flavonoid skeleton was observed with Shinoda test[11], and the violet coloration with alcoholic $FeCl_3$ gave the indication of a phenolic hydroxyl group. The UV data λ_{max}^{MeOH} 210 nm (log E 4.4), 268 nm (log E 4.22) and 335 nm (log E 4.10) was identical with that of hydroxy flavone[14]. Band-1 underwent a bathochromic shift by 48 nm in MeOH/$AlCl_3$-HCl characteristic of 5-hydroxy flavonoid[14]. Further, band-II showed a bathochromic shift by 8 nm in the presence of NaOAc indicating the presence of a free 7-hydroxy group[14]. The IR data was in close agreement with that reported for apigenin which was confirmed by Co-TLC, co-paper chromatography (table 1) and superimposable UV and IR with the authentic sample. These observations clearly suggest that the isolated compound from double recessive bz1pr aleurone tissue is apigenin.

It is known that Pr/pr controls the hydroxylation pattern and that a2pr accumulates Leucopelargonidin[4], whereas a1pr accumulates Kaempferol[8]. It was also known that the bz1pr aleurone tissue accumulates Luteolinidin[8] and the present study clearly suggests that bz1pr accumulates Apigeninidin. These studies further confirm Pr/pr controls the hydroxylation pattern of Anthocyanin B ring. The present studies also revealed that dominant CI accumulates caffeic acid and c2pr accumulates *p*-coumaric acid, r-r accumulates butein and fisetin in the known gene action sequence (figure 1). These studies clearly suggest the gene-product relationship and the control of chemical pathway in anthocyanin biosynthesis which is a classical example in higher plants like maize.

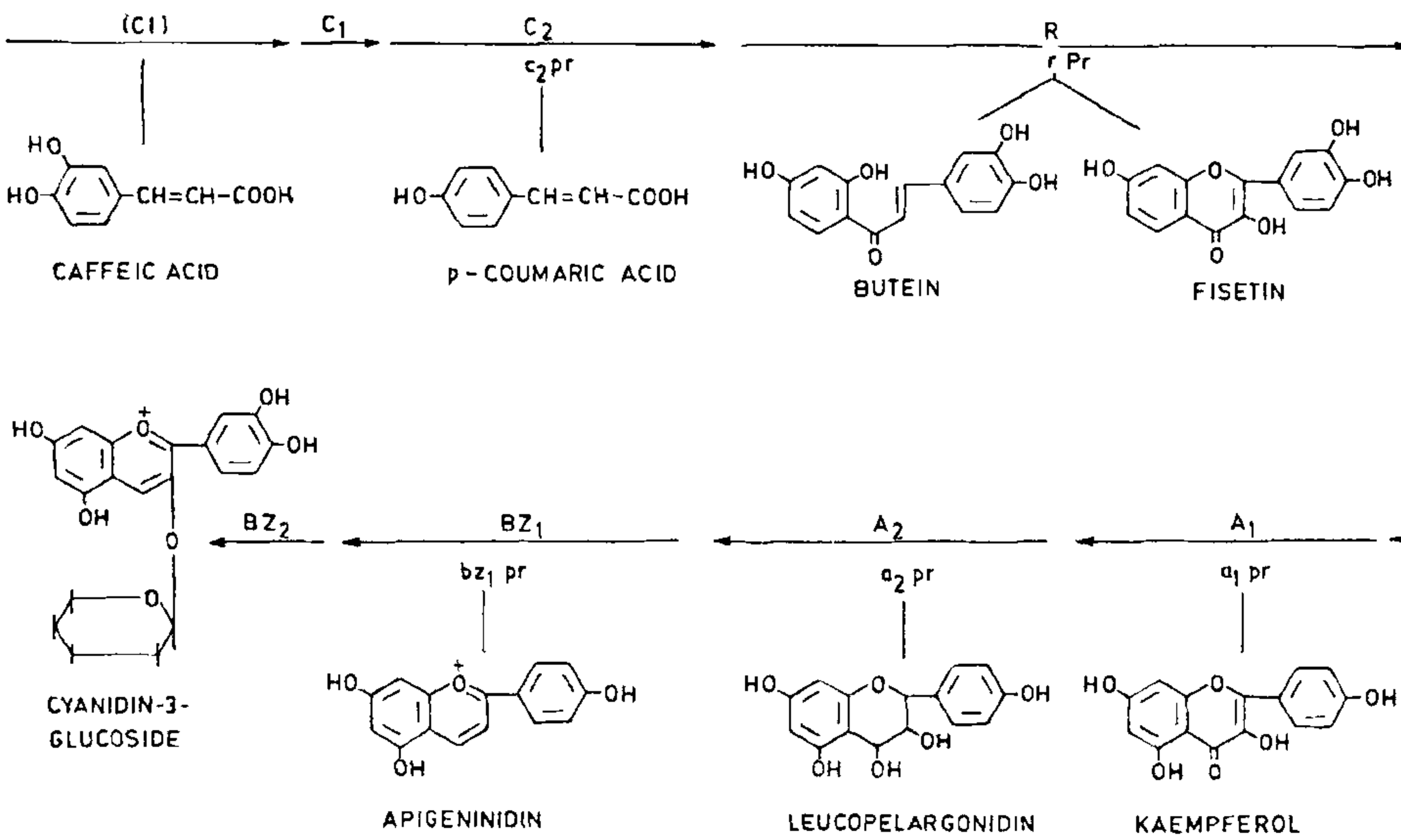

Figure 1. Gene action sequence and certain gene-controlled products in anthocyanin biosynthesis in maize.

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PHYTOPHTHORA ASSOCIATED WITH ARECANUT (*ARECA CATECHU* LINN) IN UTTARA KANNADA, KARNATAKA

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KOLEROGA or fruit rot of arecanut is one of the major diseases responsible for huge losses in the plantations of Uttara Kannada. The pathogen was first named as *Phytophthora omnivora* De Bary[1] Coleman[2] described it as *P. omnivora* var *arecae* Coleman. Pethybridge[3] considered the fungus as *P. arecae* (Coleman) Pethybridge and observed it to be quite different from *P. omnivora*. Tucker[4] reported that *P. arecae* is a synonym of *P. palmivora* Butler, which attacks many plants belonging to Palmae, whereas, Waterhouse[5] recognized it as a distinct species. Thus, controversy and confusion have existed for decades regarding the taxonomic identity and nomenclature of *Phytophthora* isolate pathogenic on arecanut. Therefore, in the present investigation, detailed taxonomic studies of *Phytophthora* isolates obtained from Koleroga-affected arecanuts of different localities were undertaken to establish the exact identity of the pathogen.

Isolates of *Phytophthora* on arecanut were collected from 25 different localities of Sirsi, Siddapur and Yellapur taluks of Uttara Kannada. The standard technique[6] was used to isolate the fungus from rotted arecanuts on PVPH medium. Single hyphal tip isolations of the isolates were made and maintained on oat meal agar.

The morphology of the isolates with reference to mycelial, sporangial, chlamydospore and oospore characteristics was studied to establish the taxonomic identity of the fungus.

The sporangial and chlamydospore characters of the isolates were studied on carrot agar. The carrot agar plates (100 mm diam) were inoculated with 7 mm diameter inoculum discs and incubated at $25 \pm 1°C$ in the dark for 3 days, after which the cultures were exposed to continuous cool light of fluorescent lamp for 2 days. The cultures were then examined for sporangial production under a microscope. For the production of chlamydospores, the cultures on carrot agar were incubated in the dark for 30 days and then observed.

The isolates were grown either singly or in combination with compatible mating types on Ribeiro's synthetic medium with β-sitosterol to obtain reproductive structures and to study their characters[7].

The characters recorded were compared with the descriptions given for different *Phytophthora* spp in the tabular key[8].

The study of different characters of the isolates revealed that in all the cases hyphae were of uniform diameter ($6\mu m$), smooth without hyphal swellings and copiously branched. Sporangia developed on carrot agar in 3–5 days, but many more developed in