

very high altitude) and an unlabeled potential group of large-eared, middle altitude flours from near Huánuco at the upper right of Fig. 1.

There are several limitations to Fig. 1, a plot based on the two most important canonical variables or axes. To properly separate the several groups in close proximity in the center of Fig. 1, at least two additional axes are needed since the distances between these groups are not well demonstrated with these two axes. By using the canonical axes here presented, one does not obtain satisfactory classification of new material unless it is closely related to one or more of the 15 groups.

Canonical analyses using other sets of variables, cob characters, characters predicted to be of "low value," and the six "best" variables, also give good separation of these groups, although the relative spacing is different. Not only does this help to corroborate the grouping presented, but it means that one can classify material using whatever variables are available.

The genetic studies are only being started. Following the suggestion of M. Goodman, the variabilities found in the F_2 generations of crosses between widely separated races will be compared with the phenotypic distances. At the same time the genetic inheritance of some important traits may be studied. It should also be possible to observe introgressive effects of one race on others.

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1. Chemical nature of the a_2 mutant.

About one kg. of a_2 and in a_2 seeds, after removal of pericarp, were defatted with petroleum ether and then extracted with cold methanol. The solvent was removed under reduced pressure and was treated with ether. Ether insolubles were separated by filtration and treated with distilled water. The aqueous solution was extracted repeatedly with ethyl acetate, and dried over anhydrous magnesium sulphate. The solvent is removed under cold conditions on a flash evaporator, which gave a

light brown substance (10 mg.). The leucosubstance thus obtained was converted to its corresponding flavylum chloride, repeatedly extracted with isoamyl alcohol, and was further purified by circular, ascending and descending chromatographic techniques with two different solvent systems, n-butanol: acetic acid: water (4:1:5) and Forestal (acetic acid 30: HCl 3: water 10).

The developed chromatograms of the crude methanolic solution of the leuco substance, after spraying with ferric reagent and subsequent heating for 30 minutes at 80°C in an hot air oven, showed a distinct green colour, whereas, with vanilin-toluene-p-sulphonic acid, they gave a strong violet red colour. The crude methanolic solution of the leuco-substance was spotted on a circular chromatographic paper and developed with the above mentioned solvent systems. The developed chromatograms, when sprayed with 10% sulphuric acid and heated, gave a characteristic purple colour. The detected spots on the chromatograms of converted anthocyanidins were eluted with 5% methanolic-HCl solution and subjected to Beckman DB spectrophotometer. The average R_f values and the absorption maxima of the converted anthocyanidin along with pure cyanidin chloride (control) are presented in Table 1.

Table 1

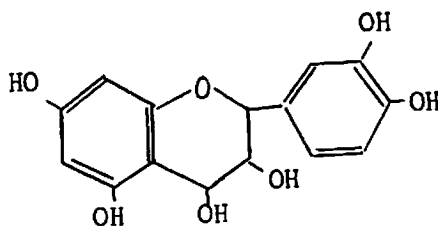
The R_f values and absorption maxima of the converted anthocyanidin and pure cyanidin chloride

Genotype	R _f values		Absorption maxima in mμ	
	BAW	FORESTAL	UV	VISIBLE
<u>a₂</u>	0.68	0.48	279	541
<u>in a₂</u>	0.68	0.47	278	542
Cyanidin chloride	0.69	0.49	279	543

In addition to the chromatographic and spectrophotometric studies, several chemical tests have been used for further characterization and confirmation of the chemical nature of the substance. The leucosubstance

gave a negative response to the "Mollish test" for carbohydrates and also some other characteristic tests for carbohydrates, suggesting that it is an aglycone. The presence of a catechol moiety in the leucoanthocyanidin is confirmed by its characteristic green colour with ferric reagent on the developed chromatogram. Its characteristic colouration with ferric chloride suggests the phenolic nature (diol 1:2) of the substance. The presence of a phloroglucinol moiety is confirmed by the characteristic strong violet red colour on heating with vanilin-toluene-p-sulphonic acid. The tests with aqueous NaOH and concentrated sulphuric acid suggested the oxygen heterocyclic nature of the compound. A rose red colour was observed when it reacted with the cyanidin reagent (1 part of cyclohexanol and 5 parts of toluene), indicating that the converted pigment is cyanidin. The conversion of the colourless leucoanthocyanidin solution to the pink coloured anthocyanidin, i.e., cyanidin, on heating with 2N HCl in a water bath for a few minutes further suggests that the compound is leucocyanidin.

Thus the chromatographic, spectrophotometric analyses, in addition to chemical tests, clearly suggest that the accumulated substance in a_2 mutant aleurones is 5, 7, 3', 4', tetrahydroxy flavan 3:4 diol (leucocyanidin).



Leucocyanidin

Preliminary studies with alcoholic extracts of various plant parts such as leafsheaths, leaves, husks, cobs and tassels of the a_2 mutant gave the indication of the presence of leucoanthocyanidin.

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