ACCUMULATION OF ANTHRANILIC ACID BY A MUTANT OF MAIZE*

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In studies on the induction of gene mutations by high energy radiation from the Bikini atom bomb, X-ray and other sources, a large number of seedling cultures have been grown for observation. Since January, 1949, maize seedling cultures have been observed by the use of filtered ultraviolet light. Normal seedlings viewed by ultraviolet light have a dull reddish-purple appearance due to the red fluorescence of chlorophyll plus the reflection of a small amount of visible blue-violet light from the lamp.[1] Several cultures, all originating from a single seed exposed at Bikini, showed segregation of a mutant type which exhibited an intense bluish-white fluorescence. In daylight no difference could be seen between the blue-fluorescent and normal seedlings. The fluorescence varies somewhat from plant to plant, both in extent and in the time or rate of development. The fluorescence increases with the age of the plants until about the four-leaf stage, then decreases and is seldom observed in plants over two feet in height. Usually the first leaf shows the most intense fluorescence. At the time of pollen shedding, anthers in the tassel fluoresce bluish-white, even more brilliantly than the seedlings. This fluorescence is localized in the anther walls. The immature pollen also fluoresces slightly, mature pollen not at all.

Chemistry.—Acetone extracts of blue-fluorescent seedlings or anthers chromatographed on $CaCO_3$ columns showed two blue fluorescent bands running some distance behind the chlorophylls. Ascending paper strip chromatograms using Whatman No. 1 paper with water saturated butanol gave much clearer resolution of blue-fluorescent components, revealing three well-defined blue-fluorescent spots. Subsequent two-dimensional chromatograms have shown up to six additional blue-fluorescent spots, fainter than the first three. The major spots were arbitrarily called A, B, and C in order of increasing R_f values. The possibility that some or all of the blue-fluorescent spots are artifacts produced in extraction was tested by chromatograms of anthers and seedlings in which the fresh plant tissue was crushed directly on the paper. The three major spots in this case appeared in the same positions as when acetone extracts were used.

A, B, and C as well as some available blue-fluorescent compounds were chromatographed in butanol-water, butanol-water with ammonia, and butanol-water with hydrochloric acid (BuOH—H_2O, BuOH—H_2O—NH_3,

and BuOH—H_2O—HCl). The R_f values so obtained are shown in Table 1. Anthranilic acid gave values similar enough to suggest identity with spot C from the maize mutant. Since *Lactobacillus arabinosus* and *Neurospora crassa* mutant B1312 are able to utilize anthranilic acid as a tryptophane substitute[2, 3] fluorescent material eluted from each of the spots was tested with these organisms. All three spots supported growth of both organisms, although only C could be anthranilic acid on the basis of chromatographic behavior.

TABLE 1

COMPARISON OF R_f VALUES OF THE MAJOR BLUE FLUORESCING SUBSTANCES IN THE MUTANT WITH OTHER BLUE FLUORESCENT SUBSTANCES

	Solvent		
Substance	BuOH—H_2O—NH_3 R_f	BuOH—H_2O R_f	BuOH—H_2O—HCl R_f
Esculin	0.35	0.38	0.45
Gentisic acid	0.11	0.32	0.88
Anthranilic acid	0.21	0.85	0.86
Mutant spot			
A	0.18	0.19	0.06
B	0.58	0.59	0.55
C	0.21	0.84	0.85

Concentrates of C eluted from chromatograms gave a positive test for primary diazotizable amine and an absorption spectrum very similar to that of anthranilic acid. A sample of blue-fluorescent tassels was used for isolation of C. One hundred grams of mutant tassels (predominantly anthers and glumes) ground to number 40 mesh was extracted three times with liter amounts of 25% acetone in water, and the combined filtrates concentrated *in vacuo* to 500 ml. The pH was adjusted to 3.5, the solution was then extracted three times with equal volumes of ether, the ether evaporated and the residue sublimed and resublimed. The white crystalline material so obtained melted at 143° (reported 144°). When mixed with a sample of anthranilic acid the melting point was 142°. An aqueous solution gave an absorption spectrum almost identical with that of anthranilic acid. The relationship of fluorescence to pH was similar to that of anthranilic acid (Fig. 1). Fraction C is thus shown to be anthranilic acid.

Assay with *Lactobacillus arabinosus* using an anthranilic acid standard curve showed that a sample of normal tassels contained the equivalent of 3.8 γ anthranilic acid per gram, mutant tassels 3650 γ per gram. Ratios of A, B, and C obtained from biological activity measurements of chromatogram eluates (expressed as anthranilic acid) were respectively 50:45:5 for a sample of mutant anthers and 16:64:20 for mutant seedlings. Bioassay of anthers of heterozygous fluorescent plants showed approximately half as much anthranilic acid activity as anthers of homozygous plants.

Discussion.—Fluorescence in ultraviolet light has been reported for a variety of plants and plant products.[4, 5] In roots of 135 species of vascular plants representing 69 families Goodwin and Kavanagh[5] found that all but 6 fluoresced in ultraviolet light, and acetone extracts of roots or rhizomes of five of the exceptions fluoresced.

In some species of *Lolium*, roots grown in contact with filter paper may secrete something that fluoresces blue. Genetic investigation has shown that this fluorescence behaves as a simple dominant trait.[6] The chemical nature of the fluorescent material was not identified.

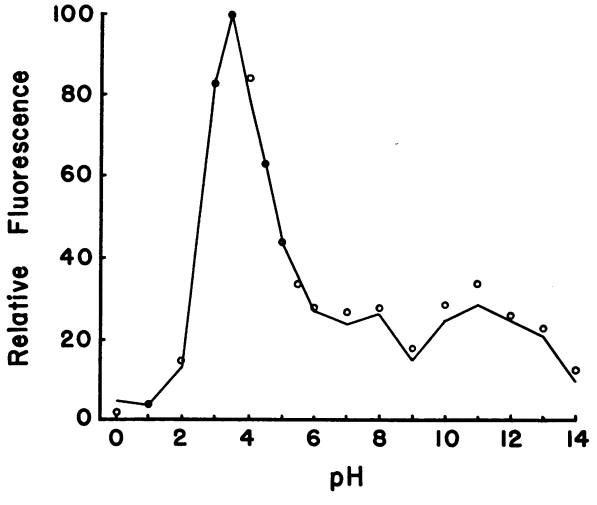

FIGURE 1

Relationship between ultraviolet fluorescence and pH for anthranilic acid (solid line) and blue-fluorescent substance C (circles). Determinations made with Coleman photofluorometer using PC-3 and PC-1 filters.

Anthranilic acid is known as a metabolite from a number of organisms. It is excreted by the cat given kynurenine and produced by slices of rat and other animal liver incubated with kynurenine or tryptophane.[7–9] It is accomulated in the medium by *Corynebacterium diphtheriae*,[10] *Bacillus subtilis*,[11, 12] certain *Neurospora* mutants[3, 13] and temporarily in the early growth of wild type *Neurospora* given large amounts of tryptophane.[3] Studies with *Neurospora* have provided substantial evidence for the existence of a cycle in which tryptophane is converted to kynurenine which is partially made into anthranilic acid that accumulates and is eventually reconverted into tryptophane through indole.[3] The nature of the changed metabolism in blue-fluorescent plants responsible for the accumulation of anthranilic acid and related substances is not known, but the mutant

plant is able to grow without exogenous tryptophane. Thus it is clear that there is no such block in tryptophane synthesis as in *Neurospora* mutant 10575.[13] The accumulated material calculated as anthranilic acid is equivalent to from 10 to 30 times the tryptophane content of the tissues. This excludes anthranilic acid production at the expense of the tryptophane level of the tissue.

Genetics.—Genetic tests have been made with fluorescent and non-fluorescent seedlings from segregating cultures. All plants which were fluorescent in the seedling stage produced fluorescent anthers. When self-pollinated, they gave progenies which were entirely blue fluorescent, although there was much variation in the amount of fluorescence. Plants

TABLE 2

CLASSIFICATION OF PROGENY FROM CROSSES OF BLUE-FLUORESCENT MAIZE

TYPE OF CROSS[a]	PROGENY NO.	SEEDLING +	SEEDLING Bf	ANTHER +	ANTHER Bf
$\frac{+}{+} \times \frac{+}{+}$[b]	2212	33	0	33	0
	2213	85	0	84	0
$\frac{+}{+} \times \frac{Bf}{+}$	1222	[c]	...	46	44
	2214	88	0	38	46
$\frac{+}{+} \times \frac{Bf}{Bf}$	2222	118	0	0	117
	2223	68	0	0	67
$\frac{Bf}{+} \times \frac{Bf}{+}$	1524	33	6	15	24
	2216	67	16	30	51
	1733	36	12	14	31
$\frac{Bf}{+} \times \frac{Bf}{Bf}$	2224	54	54	1	106
	2227	47	50	0	95
$\frac{Bf}{Bf} \times \frac{Bf}{Bf}$	2230	1	48	0	49
	2233	0	98	0	97

[a] The order of listing of parental genotypes has no significance.

[b] $\frac{+}{+}$ are normal sibs from segregating families.

[c] Seedling ratios were not obtained in progeny 1222 for plants on which tassels were scored. Subsequent tests showed 60 seedlings from the same ear were all non-fluorescent.

which developed from non-fluorescent seedling were of two types, those with fluorescent and those with non-fluorescent anthers. Selfed progenies from the plants with fluorescent anthers showed segregation of fluorescent seedlings. The plants with non-fluorescent anthers gave only normal (non-fluorescent) offspring.

In segregating cultures, the proportion of fluorescent seedlings varied from one-fourth to about one-sixth. In some cases repeated observations

showed a few plants classified as normal in the early seedling stage became fluorescent in later seedling stages. With anther fluorescence, no difficulties of classification were encountered if the anthers were well developed and producing abundant pollen. Some segregating cultures have shown a deficit of fluorescent tassels about equivalent to the deficit of fluorescent seedlings. Table 2 presents data on progeny tests of six types of crosses where observations were made on both seedlings and tassels of all plants. Only two exceptions were found, and these could be due to misclassification, stray pollination, or other sources.

Thus it appears that blue fluorescence is conditioned by a single gene. When this gene is homozygous, both seedlings and anthers are fluorescent; when heterozygous, the seedlings appear normal, while the anthers show fluorescence. In genetic studies the gene can be handled as a recessive in the seedling stage, or as a dominant in mature plants by observation of anther fluorescence. The discrepancies in ratios observed in some cultures suggest gametic selection or selective pollen tube growth, such as is frequently encountered with the genes *pr* and bt_1 in chromosome 5 (Burnham[14]) or the more clearly analyzed high and low sugary ratios studied by Mangelsdorf and Jones[15] and by Emerson.[16]

As in the above cases, some pedigrees give the expected ratios for single gene inheritance, while others give too low or too high frequencies of blue fluorescents. Tests are under way to analyze the deviations in transmission and to locate the gene for blue fluorescence.

Summary.—A mutant of maize is described in which seedling leaves and anthers of the mature plant fluoresce blue in ultraviolet light. Anthranilic acid has been identified as one of the substances responsible for the blue fluorescence. The mutant, due to a single gene, is expressed as a recessive character in the seedling and as a dominant in the anthers.

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[1] Model 90 INS ultraviolet lamp obtained from Keese Engineering Co., Hollywood, California.

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