The foregoing criteria have allowed placement of 16 new male-sterile sources and tentative placement of a 17th. The uncertainty in the latter case is due to the fact that the only diagnostic line thus far crossed with this source is N6, which produced fertile F_1 progeny. Further testing will allow placement of this source. The results of analyses of the 17 new sterile strains are presented in Table 1.

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Unusual occurrence of phlobaphenes: the non-anthocyanic co-pigments in maize embryo

Investigations on a number of R alleles have shown that the R-nj allele conditions pigmented crown and embryo (plumule). Chemico-genetical studies on the unusual presence of the pigments in the embryo could help in studying gene action. During characterization of these reddish-purple pigments it was found that, apart from anthocyanins, some other water-insoluble co-pigments of non-anthocyanic nature were also present and could be isolated from embryo tissue in ethyl acetate. The solubility of these reddish-purple pigments is ethyl acetate and their degradation during acidic hydrolysis indicate them to be non-anthocyanic. Unlike anthocyanins and anthocyanidins, the absorbance of the pigments does not decrease on standing in the unacidified solvents even after several weeks, and hence they do not undergo pseudo-base transformation. Upon exposure to ammonia vapours, the new pigments darken but do not turn blue; they are insoluble in alkali, and addition of acid results in green colour. Paper chromatographic analysis in Forestal (acetic acid-conc. HCl-water, 30:3:10 V/V) and BAW (butanol-acetic acid-water, 4:1:5 V/V, upper phase) solvent systems gave one spot near the solvent front.

Some colourless hydroxyflavans such as hydroxyflavan-3-ols and hydroxyflavan-3:4-diols are known to occur in nature. On treatment with dilute acids they are easily converted into insoluble phlobaphenes. Dehydrogenative polymerisation of polyhydroxyflavans occurs with formation of brown or red insoluble phlobaphenes, and in nature self-condensation of polyhydroxyflavans proceeds without the assistance of enzymes (non-enzymically). Also, since phlobaphenes of the 3:4-diol series may form considerable amounts of anthocyanidin under acidic conditions, their possible involvement in anthocyanin biosynthesis through 'interconversions' cannot be ruled out unless they play some sole key role in meeting other metabolic demands.

J. M. S. Mathur and N. D. Sharma

Biosynthesis of anthocyanins in maize: action spectra and role of phytochromes

Physiological studies on anthocyanin biosynthesis '*in laboratorium*' were carried out to study action spectra and phytochrome reversibility for several alleles of the R locus (R-g:Canada, R-r:Em, R-r:Standard and r-r) using red and far-red filters of predetermined absorption maxima. When exposed for a period of two days with light of restricted wavelengths in the red and far-red regions, maximum anthocyanin synthesis (determined spectrophotometrically) was found at 730 nm. Thus, wavelength dependence for anthocyanin biosynthesis in seedlings given prolonged exposures to light of approximately equal energy shows a main peak at 730 nm and a minor peak at 660 nm. These results indicate complexity of photo-control of anthocyanin synthesis, and that it is controlled by

two light steps: R-1, with an active band from about 700-780 nm and probably including some action throughout the red region; and R-2, a phytochrome mediated reaction requiring the presence of only a small amount of phytochrome in the far-red form (pfr).

The studies on action spectra suggest that light activates a flavo-protein (butyryl coenzyme A-dehydrogenase), which shows that the A ring of the flavonoid molecule is formed from 'acetate units.' It is found that increased sugar in the absence of light allows some anthocyanin formation and that leuco-anthocyanin synthesis is not light dependent. The quality of light plays an indirect role. Hence, if the leuco-anthocyanin synthesis is not light dependent and they differ from anthocyanins only in the middle (oxygenated) ring, then the light influences in some way the aliphatic chain (C-3 group) of the B-ring precursor, probably prior to cyclization. There may or may not be competition between anthocyanins and other classes of flavonoids.

J. M. S. Mathur and N. D. Sharma

Inheritance of carotenoids in maize

Investigations on the inheritance of carotenoids, the yellow pigments of maize kernels, were carried out using Parent I, orange kernels (P-I, 2904A.31) and Parent II, colorless kernels (P-II, 2360P-8) colorgraded as 18 and 1 respectively, depending upon visual intensity of pigmentation. The two parents and F_1 and F_2 populations (similarly colorgraded between 18 and 1) were analyzed for total carotenoids as well as individual component carotenoids, the latter because of the fact that dark yellow/orange color may mask the presence of light colored components and so also the dilute colored components may show diluting effect on the darker pigments.

About 25 gm of whole kernels were extracted with ether to remove any wax coating from pericarps, 20 gm dried and powdered kernels (40 mesh) extracted twice with ca.200 ml portions of hexane-acetone-water, 15:75:10 V/V, and the slurry allowed to stand overnight. The supernatant was centrifuged at 2000 xg for 15 min, passed through a column of anhydrous sodium sulphate and exhaustively extracted with n-hexane. The extract was evaporated under vacuum in a thin-film flash evaporator (bath temp. 40 C) and made up to known volume (50 ml) using n-hexane. Absorbance was measured in a 10 ml aliquot at 435 nm and 450 nm and compared with a standard β-carotene calibration curve, and total carotenoids were expressed as β-carotene (ppm).

The individual carotenoids in a 40 ml aliquot of the above extract were separated chromatographically on a magnesia column. The first fraction (polyenes) was eluted with 5% acetone in hexane followed by elution of different fractions using hexane-acetone-ethanol V/V in the proportions of 90:10:0 for zeinoxanthin, 90:10:0 for cryptoxanthin, 89:10:1 for lutein, 88:10:2 for zeaxanthin and 80:10:10 for polyoxy pigments. After diluting the different fractions, the absorbance values were determined at the appropriate wavelength: phytoene (85), phytofluene (98), β-carotene (228), zeinoxanthin (268), cryptoxanthin (216), lutein (256), and zeaxanthin and polyoxy pigments (248). To estimate major components of the polyene fraction, the values used were: phytoene (285), phytofluene (330), β-carotene (480) and total polyene fraction (425).

Total carotenoids in the colorless parent were found to be 0.4 ppm, in the orange one as high as 68.5 ppm, and in the F_1 59 ppm. In the F_2 the total pigments were in between and in varying proportions. In the F_1 all those pigments present in the parents were detected. However, the proportions of zeaxanthin, lutein, carotenes and cryptoxanthin were less than those of zeinoxanthin and polyoxy pigments. Total carotenes were found to be more in colorless (42 ppm) than in colored (6 ppm) kernels of the parents, and in the F_1 only 5.8 ppm.

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