Biosynthesis of anthocyanins in maize: presence and role of o-diphenolase and monophenolase enzymes and their isoenzyme polymorphism

During the course of chemico-genetical investigations on anthocyanin biosynthesis in maize, the cut ends of silks in many lines were found to turn brownish within a few minutes, developing a melanin type of pigment. Interest in polyphenol oxidases arises from their role in phenolic biosynthesis, oxidation of phenols and formation of dark colored pigment. By screening the genetic background of these lines it may be possible to ascertain the role of polyphenols. Polyphenols are also known to aid in pollen germination and tube growth *in vitro* and thus their presence in silks may have a more definite role in aiding pollen germination rather than in the synthesis of anthocyanins.

With this view in mind, silks from some 97 lines of different genetic background were studied for the activity of polyphenol oxidases and their isoenzyme polymorphism.

The enzyme was extracted by homogenizing 5 gm silks with 15 ml prechilled (-15 C) 0.05 M phosphate buffer (pH 6.6) and centrifuging at 20,000 xg for 20 min at 5 C. The supernatant extract was assayed immediately for polyphenol oxidase activity using catechol (10 mg/ml) and L-tyrosine (1.0 mg/ml) as substrates. The reaction mixture consisted of 2 ml catechol, 0.1 ml of enzyme preparation and 0.05 M phosphate buffer (pH 6.6) to bring the total volume to 5 ml. The mixture was incubated at 37 C for 3 min before addition of enzyme. Absorbancy was measured at 430 nm at intervals of 15 seconds. For measuring the monophenolase, 2 ml of L-tyrosine (1.0 mg/ml) solution and 0.5 ml of crude enzyme extract were used. After oxygenating the substrate for a few minutes, absorbancy was measured at 430 nm after 3 hr of incubation at 37 C. Controls consisted of everything else except substrates.

Isoenzyme studies were carried out by electrophoresis in 7.5% polyacrylamide, using tris-glycine buffer (pH 8.3). The gels were stained with L-tyrosine (1.0 mg/ml in 80% ethanol), destained and stored in 30% alcohol.

In about 10 lines no o-diphenolase activity and only negligible monophenolase activity were recorded. These lines showed no browning of cut ends of silks. In the rest of the lines definite polyphenol oxidase activity was observed, mainly the activity of o-diphenolase. The latter was also found to give 5-7 isoenzyme bands of similar nature. Unlike o-diphenolase, no multiple forms were observed for the monophenolase enzyme.

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Biosynthesis of anthocyanins in maize: in vitro tissue culture

Tissue culture of certain critical plant parts affected by each allelic form might prove useful in studies on gene action. It was found indispensable to standarize a suitable nutrient medium for culturing maize kernels *in vitro* from the early stages after anthesis, to trace the anthocyanin biosynthetic pathway based on enzyme activities and specificities. Moreover, it is possible to incorporate enzyme inhibitors or inducers in the culture medium in order to induce or inhibit the synthesis of anthocyanins.

Attempts were made to develop such a medium, and a basal nutrient medium has been standarized which gives profuse growth of maize kernels inoculated the 10th day after pollination. The medium consists of White's major elements and vitamin mixture, Nitsch's trace elements, 2% sucrose, 0.8% agar and 0.5% yeast extract (pH 6.1 - 7.0), and can be used to culture whole kernels, endosperm or embryo alone. Microscopic examination of transverse sections of kernels developed *in vitro* 20 days after inoculation revealed tissue differentiation--i.e., formation of a distinct aleurone layer.