2. Expression of pr in plant tissues.

For several years now we have been trying to develop an early inbred line of maize suitable for genetic studies. Two years ago, one family, derived initially from crosses between Gaspé plants and strains of the W22 inbred line, segregated $\underline{R}^r$ and $\underline{r}^r$, $\underline{P}^{wr}$ and $\underline{P}^{ww}$, and also what appeared from the kernel phenotypes to be Pr and pr. The $\underline{R}^r$ and $\underline{P}^{wr}$ factors were traced back to the W22 inbred strain, and the $\underline{r}^r$ and $\underline{P}^{ww}$ to the Gaspé plants. The origin of the pr factor has not been traced as yet, as the records indicate no previous segregation for red and purple seeds.

The accompanying table shows that this family has several unusual features, including:

1) A silk color factor requiring the $\underline{R}^r$ factor for the synthesis of 3-hydroxylated anthocyanins and the $\underline{P}^{wr}$ factor for synthesis of 3-deoxyanthocyanins.
2) The expression of the pr factor in such plant parts as cob, silks, and anthers as well as in the aleurone. This pr factor affects the anthocyanins, the 3-deoxyanthocyanins, the 3-deoxyleucoanthocyanidins, and the C-glycosylflavones. From the orange color of the $\underline{P}^{wr}$ pr cob, the pr factor appears to affect the P locus pigment also, probably by conditioning the formation of the precursor apiforol rather than the normal precursor luteoforol.

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3. Assay for the effects of chemical and natural substances on growth using empty endospermic sac injection.

Tonita (Proc. Int. Conf. Plant Growth Substances 1967 and 1970) has reported effects on flowering of substances injected into the empty endospermic cavity of young winter wheat seedlings. We have tested this method with maize and have used it to examine the effects of natural and chemical substances on three different stocks of maize: a) an early inbred line derived from crosses with Gaspé flint, b) an early maturing commercial hybrid, Polar Vee, and c) a strain of the comparatively late maturing inbred W_{22}.

Table 1 (Article 2)

Analysis of a family showing pr expression in the plant tissues

Cob color	red		orange		white		
Seed Color	purple	colorless	red	colorless	purple	red	colorless
Anther Color	red	red	pink	pink	red	pink	red or pink
Silk Color	red	green	orange	green	red	orange	green
Predominant flavonoids in silks							
3-hydroxylated anthocyanins	Cyanidin based	———	Pelargonidin based	———	Cyanidin based	Perlargonidin based	———
3-deoxyantho-cyanins	Luteolinidin based	Luteolinidin based	Apigeninidin based	Apigeninidin based	———	———	———
3-deoxyleuco-anthocyanidins	Luteoforol	Luteoforol	Apiforol	Apiforol	———	———	———
C-glycosyl flavones	Orientin type	Orientin type	Vitexin type	Vitexin type	———	———	———

Treatment of plants included gibberellic acid (10, 30, 100 ppm.), kinetin (10 ppm.), 3' and 5' mononucleotides (100 ppm.), and juices derived from the growing parts of the three stocks.

The procedure was as follows: Seeds were dusted with fungicide and sown individually in small plastic containers containing a mixture of soil, sand, peat moss and vermiculite. Ten days later the seedlings were taken from their containers and rinsed in tap water to remove the soil mixture from the seed. The empty seed-coat-bag stage was reached around the tenth day after planting; most of the seedlings retained the seed-coat-bag at this growth stage, and some of them still had milky endospermic liquid inside the bag. Seedlings with attached empty seed-coat-bags were selected for the investigation.

Natural and chemical substances were injected directly into the empty endospermic cavity of the seed by using a Hamilton Microlita syringe. In each case, 35.0 $\pm$ .5μl was injected into the bag. Injection was done by penetrating the empty endospermic cavity with the needle head and slowly injecting the solution.

Following injection of the chemicals and natural substances (10 plants per group), the plants were planted in a greenhouse. The plants were watered twice daily and a nutrient solution was occasionally supplied at the time of watering.

Measuring of plants was started on the 13th day after initial seeding. The experiment was terminated 60 days following seeding, at which time the Gaspé line and Polar Vee had flowered and the W_{22} was approximately half-way to maturity. Measurements were made to the closest $\pm$ .5 cm. of the newest node. In all cases two controls were included; one group was injected with 35.0 $\pm$ .5μl of distilled water and the other was grown naturally without injection.

Natural substances were prepared by squeezing out juices around the growing point of the 3 stocks to be investigated. Approximately 15 of these plants were individually homogenized in a Waring blender for one minute with 100 ml. of distilled water. The homogenized juice was filtered through a triple layer of cheesecloth three times, and then centrifuged for ten minutes at 3000 r.p.m. The supernatant was then stored at 4°C (maximum of 2 hours) until ready for use.

When this method was followed, Polar Vee responded to the gibberellic acid treatment showing increased final heights of 30%, 50% and 100% of controls with the concentrations of 10, 30, and 100 ppm, respectively. The Gaspé line showed an inverse response to concentrations, showing final heights of 100%, 60% and 6% with concentrations of 10, 30, and 100 ppm. No effects of the gibberellic acid treatment were observed with W_{22} at the time the experiment was terminated.

Kinetin enhanced the growth of Gaspé and Polar Vee but its effect was less obvious in the W_{22} variety.

All mononucleotides with the exception of cytidine 3' monophosphoric acid had some growth promoting effect. However, 5' nucleotides appeared to enhance growth more than the 3' nucleotides. Adenosine 5' monophosphoric acid and guanosine 5' monophosphoric acid had the greatest effect.

No conclusive growth differences were observed with any of the extract-injected plants.

Early indications suggest that none of these substances affects the flowering rate of maize; however, further studies are indicated.

Generally, the idea of empty endospermic sac injection seems a good one and may provide a good hormonal assay.

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1. Induction of mutants in maize pollen.

Pollen of inbred B14 was irradiated with gamma rays from a large Co^{60} source stored in the pool of the reactor at the University. The corn was grown in 1972 at the Blandy Experimental Farm in Boyce, 90 miles away. Pollen was collected early in the morning, brought to Charlottesville for irradiation and returned to Blandy, where sib pollinations were made the same day. The pollen was irradiated in a container lowered into the water surrounding the Co^{60} source to a depth determined to give an exposure of 1300 r. This was the most common