

Some Physiological Effects of Viviparous Genes vp_1 and vp_5 on Developing Maize Kernels¹

Received for publication April 19, 1973

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ABSTRACT

The effects of two viviparous genes, vp_1 and vp_5 , on development of the maize (*Zea mays* L.) embryo and endosperm were investigated. Differences between viviparous and normal embryos first appeared at 25 to 30 days after pollination. Increases in fresh weights indicated that viviparous began to grow more rapidly than normal embryos at that time. Amino acids and ethanol-soluble carbohydrates also accumulated more rapidly in viviparous, but a reserve material (lipid) was lower in viviparous than in normal embryos.

The fresh and dry weights and total nitrogen content of endosperms from viviparous resembled those of normal seeds until about 30 days after pollination, but were all lower in viviparous after that time. Pronounced differences in α -amylase activity were not observed until late in development (40 days after pollination) when the enzyme increased in viviparous seeds only. Developmental changes in viviparous seeds generally resemble those of normally germinating seeds.

Vivipary or premature germination in maize (*Zea mays* L.) is expressed by the development of seedlings from immature embryos while still attached to the parent plant. At least eight recessive mutants that can induce vivipary separately in maize have been identified and their respective genotypes described in detail (7, 19, 20, 22). In most cases viviparous genes are associated with other expressions besides premature germination (5-7, 16, 18-22). Most noticeable is their effect on plant pigments. Five of the eight viviparous genotypes suppress carotene formation (22) with the result that chlorophyll also fails to develop in the seedlings (12, 13, 25). A sixth gene (vp_1) suppresses the development of anthocyanin pigments in the aleurone layer but does not affect carotene or chlorophyll development (22).

Robertson (22) suggested that vivipary and inhibition of pigment expression are caused by pleiotropic action of a single gene, but the nature of the metabolic blocks involved or how a block in pigment synthesis might induce vivipary is unknown.

Abscisic acid is thought to prevent precocious germination of cotton embryos (9), and this compound may also be a dormancy factor in maize seeds. The possibility of a common

metabolic pathway for carotenoids and abscisic acid (24), suggests the manner in which a single metabolic block might induce both carotene deficiency and vivipary. Also a single block might simultaneously reduce anthocyanins and metabolically related germination inhibitors such as cinnamic acid, caffeic acid, or coumarin.

The multiplicity of viviparous maize mutants indicates the complexity of the mechanism controlling developmental dormancy (18, 22), and it is possible that several dormancy factors must be present to prevent premature germination. Because embryo growth was obtained on artificial medium after removal of embryos from developing maize seeds, there were early suggestions that factors responsible for embryo dormancy are produced in the endosperm (8, 15). If dormancy-inducing factors are produced by normal endosperms, then genetic evidence indicates that viviparous embryos may be insensitive to such factors; the genotype of the embryo dictates whether viviparous development will occur (21).

Studies of the metabolic basis of viviparous genes should lead to a better understanding of the mechanisms that normally prevent precocious germination. The present work was undertaken as a step toward this goal. The present study was concerned with the timing of the onset of viviparous growth in relation to mobilization of reserve materials and with nutritional relationships between endosperm and viviparous embryos. The developmental patterns of two viviparous mutants were studied: vp_5 , a typical carotene-deficient mutant, and vp_1 , the anthocyanin deficient mutant.

MATERIALS AND METHODS

The viviparous mutants used in this study were obtained from the Maize Genetic Cooperative, Department of Agronomy, University of Illinois, Urbana. Mutant vp_1 produces normal-looking plants which produce only viviparous kernels when self-pollinated. This mutant also suppresses development of aleurone anthocyanin pigments associated with A_1 , A_2 , C, R, and i genes (22). This suppression serves as a good marker for identifying vp_1 kernels on heterozygous plants (Fig. 13). Such aleurone pigments are not visible until 25 days after pollination. Therefore, this criterion cannot be used before that time. Homozygous vp_1 plants were obtained from dormant seeds from heterozygous ears. These seeds were planted in the greenhouse, and the plants self-pollinated. At 35 DAP,³ viviparous kernels with actively growing seedlings were removed from the cobs, planted in flats, and later transplanted to the field, when the seedlings were about 6 inches tall. Later, it was found that vp_1 seeds would grow if planted directly in the field.

¹ This work was supported by funds from the University of Illinois Agriculture Experiment Station.

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³ Abbreviation: DAP: days after pollination.

Mutant vp_6 suppresses development of carotene and prevents development of chlorophyll. Kernels of vp_6 were obtained from heterozygous plants. They could be identified by their whitish color, in contrast to the yellowish color that develops in normal kernels after about 15 DAP (Fig. 14).

Sampling for vp_1 began at 10 DAP and continued at 5-day intervals until 45 DAP. Sampling for vp_6 began at 15 DAP and continued at 5-day intervals until 40 DAP. Slow embryonic development in vp_1 made it difficult to separate embryo from endosperm before 20 DAP. Embryos of vp_1 at 20 DAP showed about the same degree of development and ease of separation as embryos of vp_6 at 15 DAP. The data presented here cover only the period when embryos and endosperms could be studied separately.

Ears were harvested at mid-day and taken to the laboratory where they were processed immediately or stored at -40 C for later use. Each sample consisted of three replicates (three different ears), with the kernels being taken from the middle of the ears.

After careful removal of the remains of silks and glumes, the embryo was dissected from the endosperm. The pericarp was removed from the endosperm, and alcohol extracts were prepared as described below.

Fresh and Dry Weights. Fresh weight was measured immediately after dissection. Dry weight was measured on the same sample after drying at 65 C for 24 hr in a forced draft oven.

Total Nitrogen. Total nitrogen was determined on dry tissues according to Lang (14). The following modifications were made. After the digests had cooled, 0.9 ml of H_2O was added. Aliquots of 0.1 or 0.2 ml were made up to 9.5 ml with 0.5% Duponal (E. I. duPont de Nemours & Co.). Each sample was thoroughly mixed with 0.5 ml of Nessler's reagent. After allowing 10 min for color development, the absorbance was read at 430 nm on a Beckman DB-G spectrophotometer.

Alcohol Extracts. Alcohol-soluble metabolites were determined on alcohol extracts prepared by dropping 10 to 20 fresh embryos or endosperms in boiling 95% ethanol. After cooling, the plant parts were ground in a mortar or conical glass homogenizer. The homogenate was cleared by centrifugation, and the supernatant fraction was collected. The pellet was washed with more ethanol, centrifuged, the second supernatant fraction was added to the first, and the volume was adjusted.

Amino Acids. α -Amino nitrogen was determined from the alcohol extracts by the method of Yemm and Cocking (26). The values were expressed as amino acids.

Soluble Carbohydrates. Total soluble carbohydrate was determined from the alcohol extracts by the anthrone procedure described by Johnson *et al.* (11). The results are expressed as milligrams of glucose.

Lipids. The method of Bligh and Dyer (1) was used for determining embryo lipid content. Fresh tissue was used.

Amylase. Amylases were extracted from whole kernels by the method described by Dure (4). Ten kernels were homogenized with ice-cold double-distilled water in a chilled mortar and brought to a final volume of 20 or 30 ml. The homogenate was centrifuged (12,000g, 15 min, 0 C), and the supernatant fluid was assayed for α -amylase activity according to Chrispeels and Varner (2). Enzyme activity was expressed as mg starch hydrolyzed/min·volume of extract equivalent to one maize kernel, *i.e.* decrease in starch capable of forming the colored iodine complex.

RESULTS

Fresh and Dry Weights. The rates of development of the various embryos with respect to fresh and dry weights are

shown in Figures 1 and 7. The fresh weight of vp_1 embryo (Fig. 1) increased rapidly from 6 mg/embryo at 20 DAP to 149 mg at 45 DAP, whereas the related normal embryo increased from 7 to 58 mg. The fresh weight of vp_6 embryo (Fig. 7) was similar to that of normal embryo until after 25 DAP, then vp_6 embryo increased rapidly to 171 mg at 40 DAP whereas the normal embryo increased to 48 mg. The dry weights of viviparous embryos did not reflect the higher values shown by the fresh weights. Generally the dry weight of the viviparous embryo was lower than that of normal embryo of the same age (Figs. 1 and 7).

The changes in fresh and dry weights of the various endosperms are shown in Figures 2 and 8. In both strains, fresh and dry weights of viviparous endosperms were generally lower than those of normals, and the patterns of development were similar, except for the decline in vp_6 endosperms towards the end of the period.

Moisture Content. The difference in moisture content observed between vp_1 and its related normal embryo (Fig. 3) indicates that two different forms of development were taking place. Moisture content of the vp_1 embryos increased slightly (from 75–80%) from 20 to 45 DAP, whereas normal embryos decreased from 75 to 50% during the same period. The moisture content of vp_6 and related normal embryos declined together initially, and reached 65% at 25 DAP. Thereafter, the normal embryo continued to decline, but vp_6 decreased no further (Fig. 9).

The moisture contents of both viviparous and their related normal endosperms showed similar developmental patterns (Figs. 3 and 9). Although viviparous embryos were absorbing a larger amount of moisture, their endosperms did not lose moisture more rapidly than did normal endosperms.

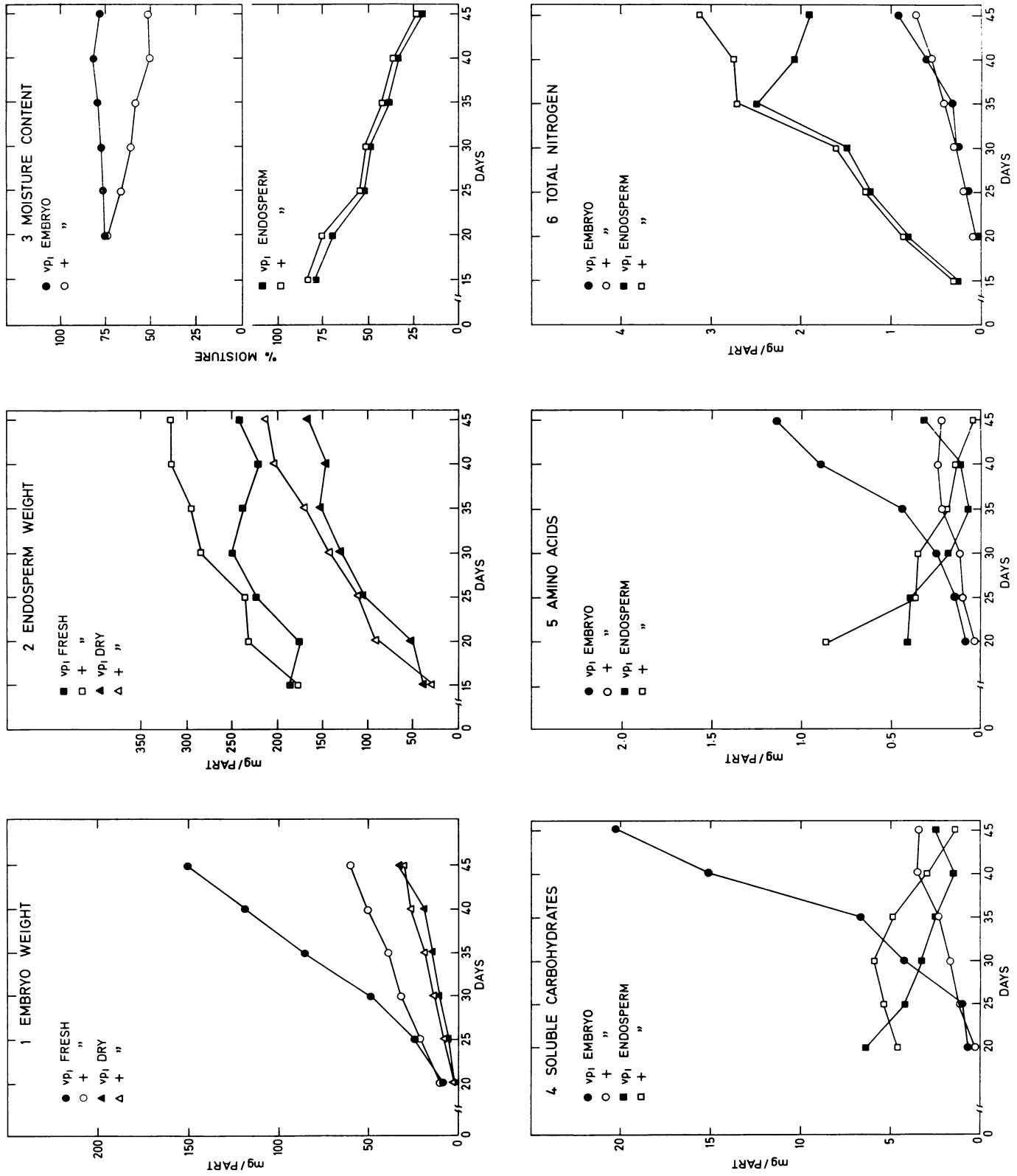
Amino Acids. In general, viviparous embryos had higher free amino acid contents. As early as 20 DAP, the amino acid content was higher in vp_1 than in the normal embryo. This difference increased with time until, at 45 DAP, vp_1 embryos contained five times more amino acid than did normal embryos of the same age (Fig. 5). Similar developmental changes were observed for vp_6 embryos (Fig. 11).

In vp_1 endosperm, the amino acid content declined from 0.4 mg/endosperm at 20 DAP to 0.07 mg at 35 DAP, then rose steadily to 0.3 mg at 45 DAP (Fig. 5). The increase in amino acid content occurred as total nitrogen decreased (Fig. 6) and may be due to increased protease activity. Similar trends for amino acid content were observed in vp_6 and related normal endosperms up to 35 DAP, then vp_6 increased, whereas the normals did not (Fig. 11).

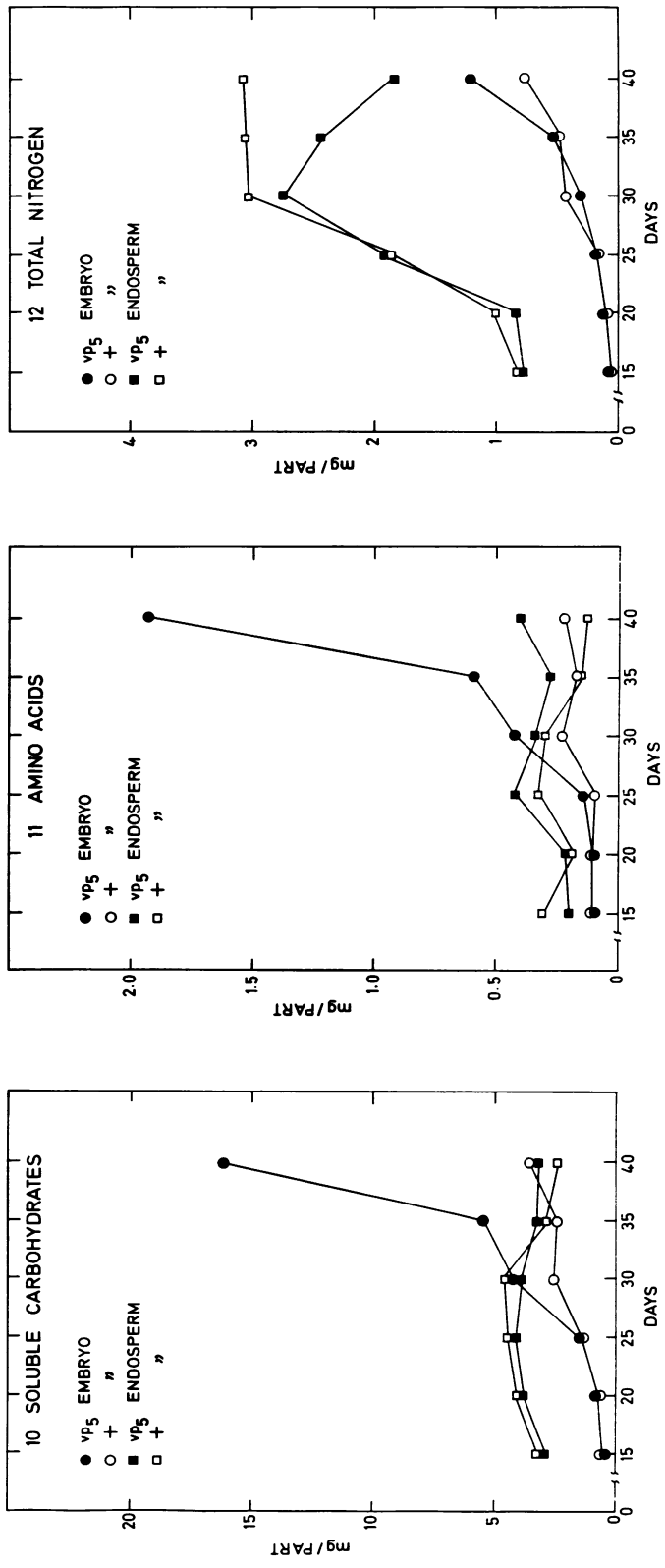
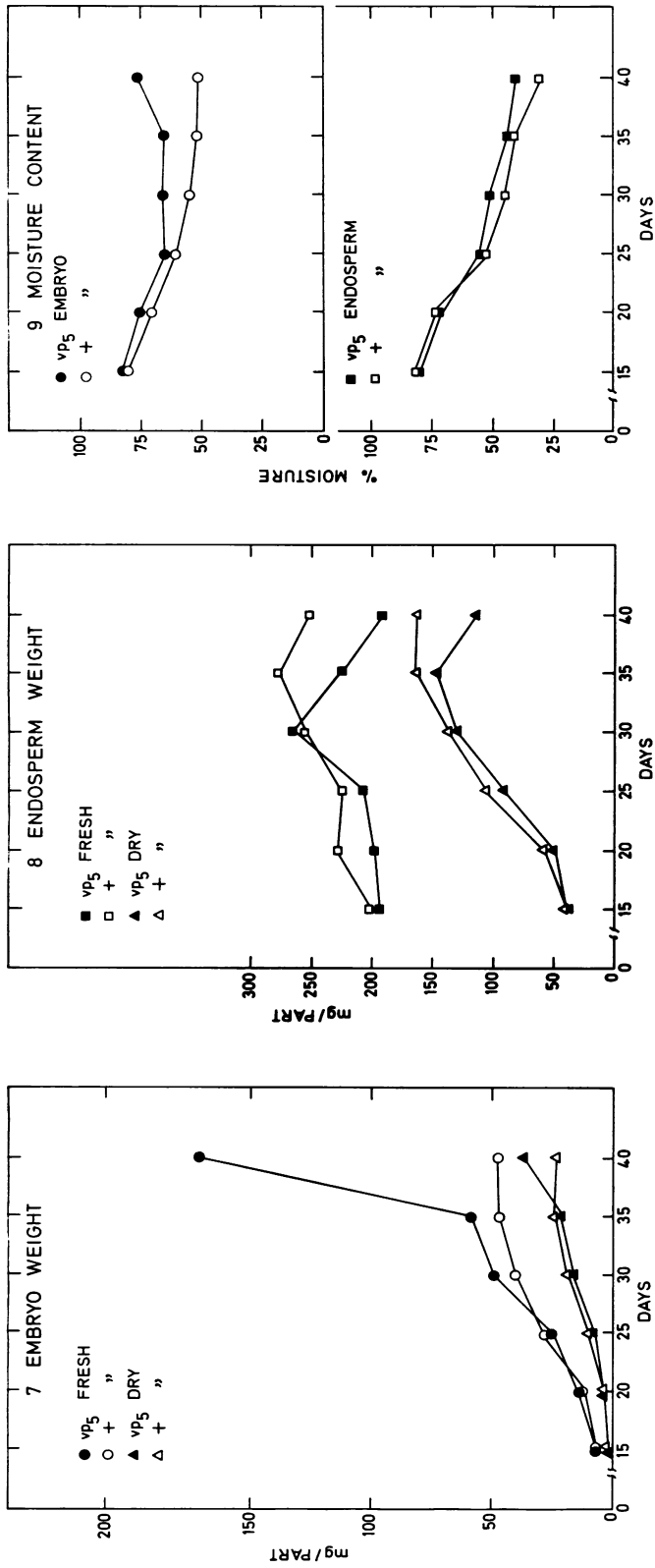
Total Nitrogen. Developmental patterns for embryos and endosperms with respect to total nitrogen are presented in Figures 6 and 12. Total nitrogen of both vp_1 and its related normal embryos increased at the same rate between 20 and 30 DAP, then the rate of increase became slightly higher for the viviparous embryo. From 20 to 35 DAP, total nitrogen accumulated rapidly in endosperms of vp_1 and the related normal genotype. Subsequently, there was a net loss of nitrogen from the vp_1 endosperm only, and this loss coincided with increased nitrogen accumulation by vp_1 embryos. Changes in total nitrogen for vp_6 embryo and endosperm (Fig. 12) were similar to those observed for vp_1 .

Soluble Carbohydrate. The soluble carbohydrate contents of the various embryos and endosperms are shown in Figures 4 and 10. The developmental pattern is similar to that described above for amino acids—increased carbohydrate associated with growth of viviparous embryos and a tendency toward decreased carbohydrate in viviparous endosperms compared to normals.

Embryo Lipids. The lipid content of vp_1 remained relatively



Figs. 1-6. Changes in weight, moisture content, soluble carbohydrates, soluble amino acids, and total nitrogen in developing viviparous (*vp₁*) and related normal (+) maize embryos and endosperms.



Figs. 7-12. Changes in weight, moisture content, soluble carbohydrates, soluble amino acids, and total nitrogen in developing viviparous (vp₅) and related normal (+) maize embryos and endosperms.

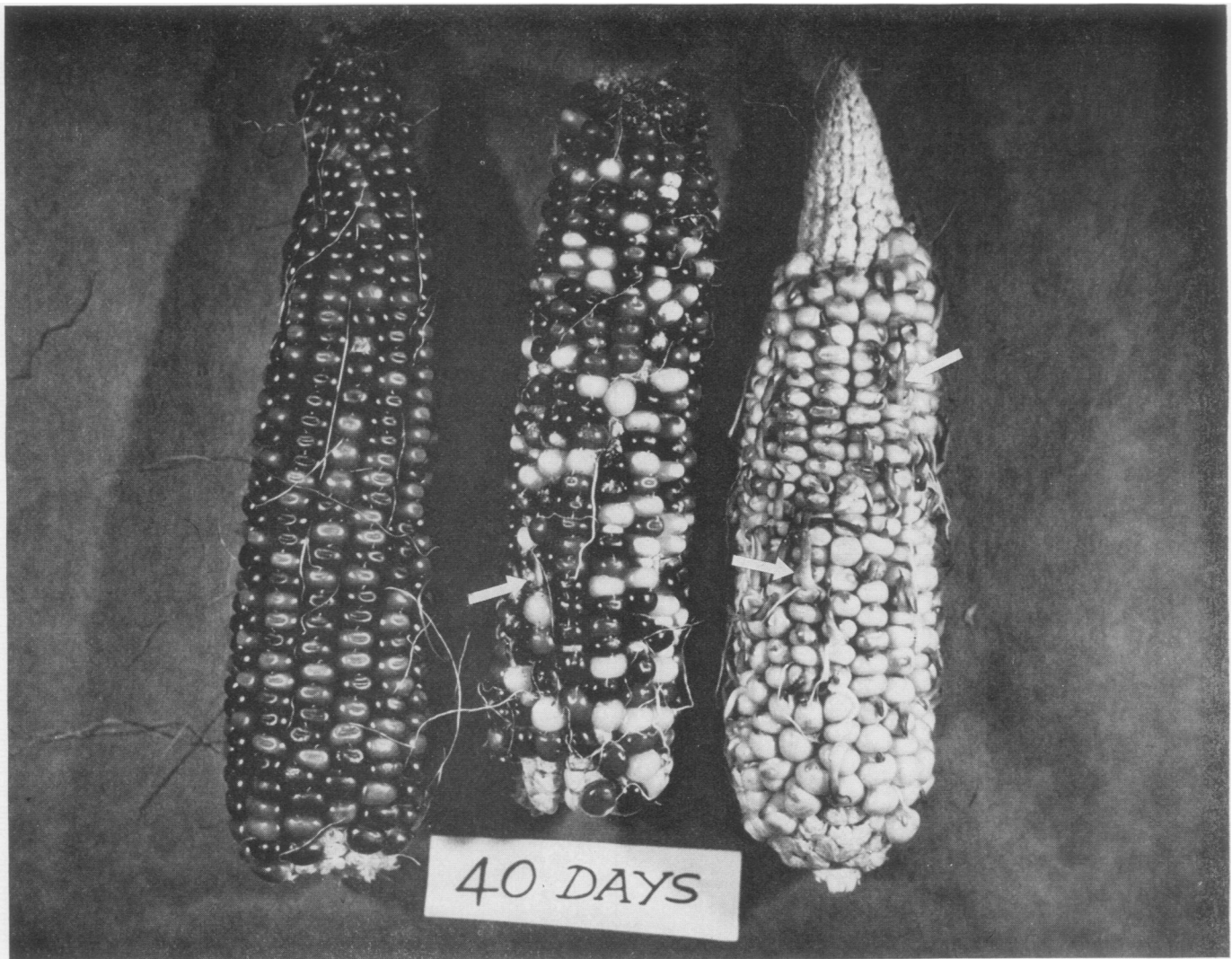


FIG. 13. Cobs from lines carrying vp_1 . Left: ear with normal dormant kernels showing red or purple aleurone pigments; center: segregating ear with red or purple dormant kernels and yellow homozygous vp_1 kernels; right: self-pollinated ear from homozygous vp_1 plant, all kernels lack red or purple aleurone pigments. Many viviparous seedlings (indicated by arrows) have broken through the pericarp.

low and constant at about 1.7 mg/embryo during most of the developmental period, with a decline to 0.9 at 45 DAP (Table I). In contrast, the lipid content of the normal embryo increased steadily from 3.1 to 9.7 mg/embryo over the same period. The vp_5 embryo also contained less lipid than its normal relatives (Table I), but the difference was not as pronounced as that observed between vp_1 and normal embryos.

Amylase. The α -amylase activity of normal kernels remained constant or declined during the period 30 to 40 DAP (Table II). In contrast, α -amylase of viviparous mutants increased markedly from 35 to 40 DAP, an increase which coincided with the maximum rate of embryo sugar accumulation.

As sampling progressed, it was evident that depression of anthocyanin formation was a reliable indicator of vivipary in vp_1 . Only rarely did yellow homozygous vp_1 kernels fail to germinate prematurely. Many of the white homozygous vp_5 kernels, however, became dormant, a possible result of the incomplete penetrance exhibited by this mutant (22), perhaps due to premature moisture loss (22). Accordingly, vp_5 data for 15 and 20 DAP must be interpreted with caution. Carotene deficiency was the sole criterion at these early stages, so the

samples were probably a mixture of dormant and viviparous kernels.

DISCUSSION

Vivipary, like normal germination, is a process that gives rise to seedling development, so the physiological changes associated with these two processes should be similar. The results presented above support this assumption; physiological changes in the viviparous embryos are similar to those reported for normally germinating embryos (3, 4, 10, 17). In particular, changes in soluble carbohydrates and amino acids resemble those reported for normally germinating kernels (10), except that the viviparous embryo begins development at a time when the endosperm is rich in soluble nutrients. The higher levels of soluble components observed in viviparous embryos could, therefore, result from more rapid absorption of such components from the endosperm, causing the decrease in amino acids and soluble carbohydrates observed for vp_1 endosperm over the 25 to 40 DAP period. Immediate utilization of translocated metabolites by viviparous embryos would

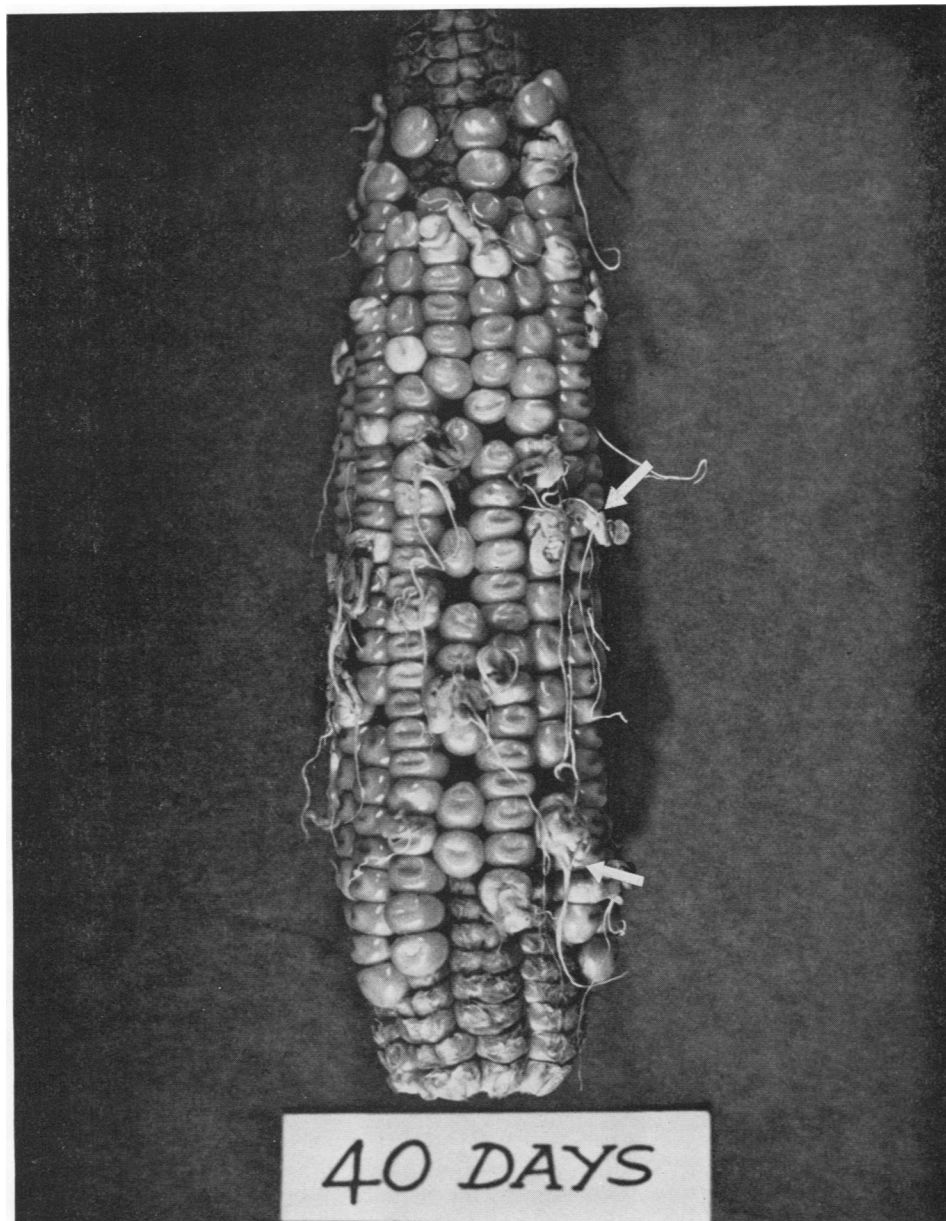


FIG. 14. Cob segregating for the viviparous gene *vp_s* showing actively growing seedlings at 40 days after pollination and white kernels, another expression of the gene. Arrows indicate viviparous seedlings which have broken through the pericarp.

Table I. Lipid Content of Developing Maize Embryos

Age of Embryos	Lipid Content ¹			
	<i>vp₁</i>	+	<i>vp_s</i>	+
<i>days after pollination</i>	<i>mg/embryo</i>			
20			0.9	1.2
25	1.7	3.1	3.4	3.9
30	1.7	5.9	4.7	5.9
35	1.6	6.8	4.3	7.4
40	1.7	9.6		
45	0.9	9.7		

¹ *vp₁* and *vp_s*: viviparous mutants; +: corresponding normal genotypes.

Table II. Amylase Activity of Developing Maize Kernels

Age	Amylase Activity			
	<i>vp₁</i>	+	<i>vp_s</i>	+
<i>days after pollination</i>	<i>mg starch hydrolyzed/kernel·min</i>			
15	0.13	0.13	0.23	0.19
20			0.45	0.45
25	0.32			
30	0.47	0.48	0.51	0.59
35	0.47	0.41	0.74	0.35
40	1.20	0.38	1.58	0.24

delay the need for hydrolytic enzymes to mobilize insoluble endosperm reserve materials. Hence, the observed late increase in α -amylase did not delay viviparous growth.

Embryo lipids are major metabolic substrates during the early part of normal maize germination (3, 10). However, lipids are probably not important substrates for early viviparous embryo development unless their low lipid content results from rapid synthesis and breakdown. It is more likely that incoming carbohydrates are diverted away from lipid synthesis and channeled directly into biosynthetic pathways associated with growth.

The generally higher moisture content of viviparous embryos was probably not a causal factor but was a consequence of vivipary. Viviparous and normal embryos had similar moisture contents in the early stages of development when changes pertinent to vivipary were probably initiated. Also, exposure to moisture does not induce germination in immature normal kernels (19, 24).

The possibility of hormonal differences between viviparous and dormant embryos was also considered. Preliminary experiments with the wheat coleoptile straight growth bioassay were conducted to learn whether viviparous embryos contained lower levels of growth inhibitors or higher levels of growth-stimulating substances, but no such differences were detected (25). Whether viviparous embryos are relatively insensitive to growth inhibitors has not been established, nor is it known whether vivipary is associated with altered levels of cell division factors. Viviparous growth begins as early as 20 days after pollination, but altered rates of cell division might occur even earlier.

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