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Analysis of Glucose Metabolism in the Heterotic Viability in Seedling Growth of Maize F_1 Hybrid

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Abstract : We have analyzed seedling growth of maize F_1 hybrids, CO_2 evolution from kernels and glucose metabolism in embryonic cells during the germination process, and compared these factors with equivalent factors for their parental inbred lines. The heterotic F_1 hybrid (Oh545×W22) germinates and grows faster than parental inbred lines. The evolution rate of CO_2 from germinating kernels was also higher in the heterotic F_1 hybrid. However, these trend were not seen in the non-heterotic F_1 hybrid (Oh545×Oh45). Glucose metabolism in the embryonic cells was evaluated by *in vivo* labelling of CO_2 and cellular components with [U-^{14}C] glucose. In the heterotic F_1 hybrid, the assimilation rate of radioactivities from [U-^{14}C] glucose into amino acids, proteins, nucleic acids, organic acids and CO_2 was significantly higher than that of the parental inbred lines after 24 hrs of germination. These results show that quick activation of metabolic function in the embryo after the onset of water uptake is due to the heterotic F_1 hybrid genotype, and is one of the key factors in the mechanism by which the heterotic F_1 hybrid expresses its hybrid vigor in the germinating process.
Key words : F_1 hybrid, Germination, Glucose metabolism, Hybrid vigor, Maize, Seedling growth.

トウモロコシ F_1 雑種の芽生え成長時に発現する雑種強勢に関連したグルコース代謝の解析 : 三野真布・*井上雅好（全農農業技術センター・*京都府立大学農学部）

要　旨 : 草丈，種実収量などで雑種強勢を強く発現するトウモロコシ F_1 雑種 (Oh 545×W 22) と発現しない F_1 雑種 (Oh 545×Oh 45) の間で芽生えの成長速度，発芽種子からの二酸化炭素ガス (CO_2) の排出速度を比較調査した．その結果，F_1 雑種 Oh 545×W 22 は芽生えの成長速度，CO_2 排出速度共に，その両親系統だけでなく他方の F_1 雑種 Oh 545×Oh 45 やその父本の Oh 45 をも陵駕することを認めた．一方，Oh 545×Oh 45 は両親系統の成績と大差なかった．F_1 雑種の遺伝子型の違いが，芽生え成長速度や CO_2 排出速度におよぼすこのような影響から，雑種強勢が種子発芽直後から既に発現することを確認した．次に代謝生理からこの現象をとらえる目的で，種子発芽時の呼吸に重要な役割を果たす基質が糖であることから，[U−^{14}C] グルコースを F_1 雑種 Oh 545×W 22 とその両親系統の芽生えに取り込みこませその動向を検討した．吸水開始後の様々なステージで取り込んだグルコースが有機酸，アミノ酸，核酸，タンパク質，細胞壁構成成分，CO_2 に代謝される速度を調査した．放射能の各画分への取り込みは，種子吸水開始 24 時間目から F_1 雑種において両親よりも有意に高くなることから，吸水後の胚の代謝機能が F_1 において両親よりも早く高まることを確認した．そして F_1 雑種のこの代謝機能の特徴が，芽生えの成長期での雑種強勢発現の重要な要因の一つであると結論した．
キーワード : F_1 雑種，グルコース代謝，雑種強勢，トウモロコシ，発芽，芽生えの生長．

Consideration of hybrid vigor is one of the most powerful strategies in crop improvement, especially for cross-pollinating crops such as maize, sorghum etc. as well as for improving some self-pollinating crops, eg. rice[6]. The mechanism by which the F_1 hybrid genotype shows the superiorities in growth and yield over its homologous parents have not been clarified genetically. However, there are much evidences to suggest that F_1 hybrid plants are more active in terms of the physiological and biochemical functions related to cell growth[8].

As a part of the studies on the early manifestation of maize's hybrid vigor, we had been analyzing metabolic activity in germinating embryos of the F_1 hybrid. The maize's F_1 hybrid showed a higher level of activities in nucleic acids and protein synthesis, and in enzymatic activities related to lipid metabolism and protein degradation, than do the parental inbred lines[9)-13)]. These studies indicated that the active cellular metabolism in F_1 hybrid embryo should be considered for the analyzing heterosis.

Ideally, major metabolic activity in the cells could be evaluated in terms of the glucose metabolism, which generates energy and metabolites indispensable for cell growth. Notably, it has been indicated that the substrate consumed in respiratory metabolism during the

early stages of germination is sugar[7]. To this end, using [U-^{14}C] glucose as a tracer, we investigated the glucose metabolism in germinating embryos of maize F_1 hybrid in comparison with its parental inbred lines.

Materials and Methods

1. Plant materials

The inbred lines, Oh545, W22, Oh45 and their F_1 hybrid lines (Oh545×W22 and Oh545×Oh45) were used. In the field experiments, we have designated Oh545×W22 as the heterotic F_1 hybrid and Oh545×Oh45 as the non-heterotic F_1 hybrid[11]. Since non-heterotic phenomenon is also appeared in the germinating stage of Oh545×Oh45, we used this F_1 hybrid as a negative control for the determination of seedling growth and CO_2 evolution. All kernels used in the present experiment were harvested from plants cultured in the same field during the same year, and cold-stored (4°C) in a desiccated condition. The experiments for germination and feeding of [U-^{14}C] glucose to the kernels were conducted under the aseptic conditions.

2. Measurement of CO_2 evolution from the germinating kernels

Five kernels collected at each sampling time were transferred into a flask (50 m*l* in volume), then incubated at 30°C for 30 min in darkness after sealing each flask with a rubber plug. The air was sampled both at the start and the end of incubation, and net CO_2 evolution rate was determined as the difference between these two samples. Concentration of CO_2 was measured with a gas chromatograph (Hitachi 663-50) using a thermal conductivity detector[1].

3. Feeding of [U-^{14}C] glucose

Germinating kernels were transferred in a glass vessel, in which a filter paper wick adsorbing 0.5 m*l* of potassium hydroxide solution (300gL^{-1}) was placed in an isolated central well. Feeding was started by adding 5 m*l* of 1 μCi/m*l*D-(U-^{14}C)-glucose (269.9 mCi/mmol, RCC, Amersham) solution. The culture were incubated at 30°C in the dark for 1-2 hrs, terminated by adding 1 m*l* of trichloro acetic acid solution (350 gL^{-1}) to the vessel, and kept for 1 hr to complete the CO_2 transfer to the filter paper. In order to determine the CO_2 evolution rate, the radioactivity adsorbed on filter paper was counted on liquid scintillation spectrometer (Packard Tri-Card 3380), by using a dioxan scintillator system. Embryos excised from the kernels were collected and stored −20°C until use.

4. Extraction and determination of cellular components in embryo tissue

Each cellular component (amino acids, organic acids, sugar, nucleic acids, proteins and residual fraction) of embryo tissue was extracted by a modified methods of Ishikawa et al.[5] as shown in Fig. 1. ^{14}C-activities in each fraction were determined by the same method mentioned above.

5. Determination of embryo dry weight

For each sampling stage one hundred kernels were germinated under the same conditions. Embryos were excised, dried and weighed for obtaining representative values of embryo dry weight at each germination stage.

6. Statistical test

Comparisons of the means were made by a Q test following performance of single factor ANOVAR[14].

Results

1. Seedling growth and CO_2 evolution

The seedling growth rate evaluated by embryo dry weight at each germinating time were shown in Table 1. Up to 24 hrs of imbibition, there was no apparent difference for the growth rate among the lines. However, heterotic F_1 hybrid, Oh545×W22, showed more vigorous growth during next 24 hrs than other lines examined. The growth index at each time obtained on the basis of embryo dry weight at 6th hr as 100 indicated this tendency more clearly; the value at 48th hr was 133 for heterotic F_1 hybrid, while 110 for the parental lines. On the other hand, the non-heterotic F_1 hybrid, Oh545×Oh45, did not show any vigor in terms of seedling growth (cf. growth index at 48th hr is 107) (Table 1). These results indicate that the heterotic F_1 hybrid genotype leads active cell growth in the embryo tissue during germination.

To test whether this phenomenon is directly related to cell metabolic activity, the CO_2 evolution rate was evaluated. The results are shown in Table 2. After 16 hrs of imbibition, the amount of CO_2 evolved from the heterotic F_1 hybrid significantly exceeded that evolved from the parents. However, as expected, this

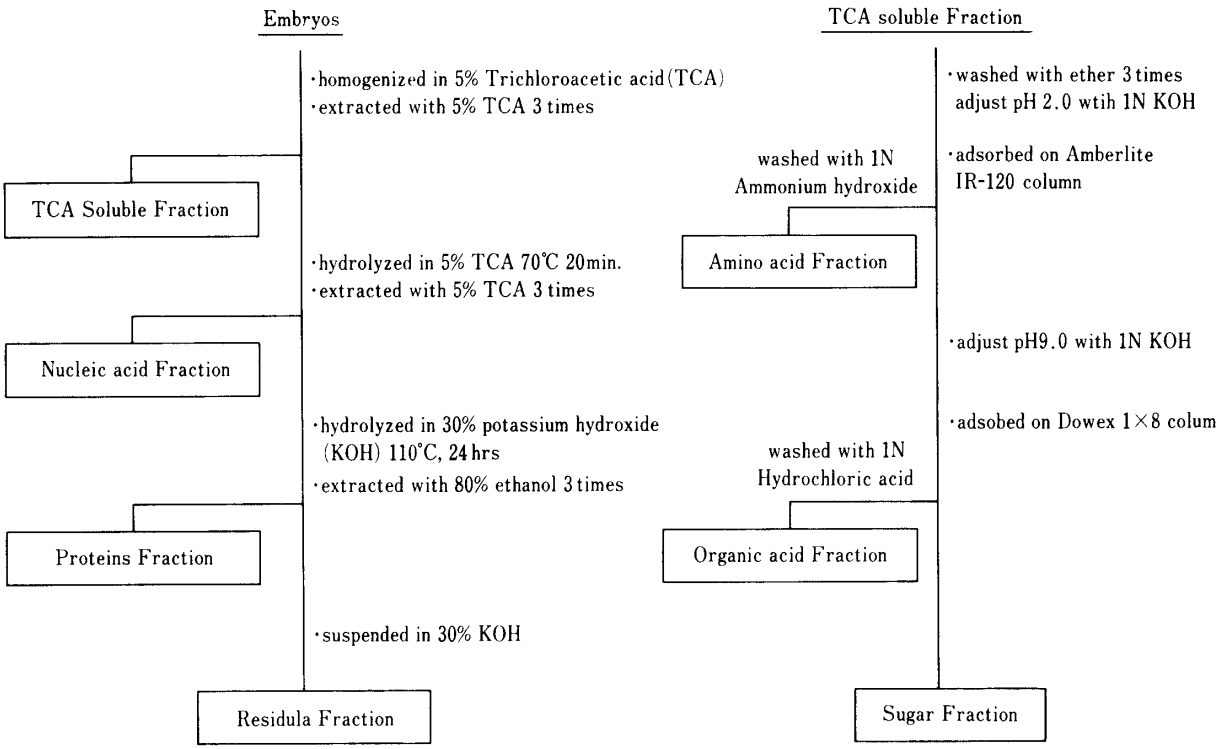

Fig. 1. Diagram of extraction procedure for cellular components in the embryo tissue.

Table 1. Embryo dry weight of germinating kernels of F_1 hybrid lines and their parental inbred lines.

Time after imbibition (hr)	Embryo dry weight (mg. D.W. embryo^{-1})				
	Oh545	W22	Oh45	Oh545×Oh45	Oh545×W22
6	30 (100)*	28 (100)	30 (100)	30 (100)	30 (100)
12	30 (100)	27 (96)	29 (90)	30 (100)	30 (100)
24	31 (103)	27 (96)	30 (100)	30 (100)	32 (107)
36	32 (107)	29 (104)	31 (103)	31 (103)	33 (110)
48	33 (110)	31 (110)	33 (110)	32 (107)	40 (133)

* : Data in parenthesis are index values (%) of embryo dry weight at each germination stage on the basis of dry weight at the 6th hr as 100.

phenomenon was not seen in the non-heterotic F_1 hybrid. Although we did not measure the oxygen uptake of the kernels, the present results suggest that the heterotic F_1 hybrid increased its respiratory activity rapidly as compared to other lines. This high level of respiratory activity would tend to consume more substrate in the cells, and concomitantly make the cells synthesize various precursors indispensable for the process of building up cellular components more vigorously.

2. Incorporation of [U-^{14}C] glucose into CO_2 and cellular components

In order to directly analyze the higher state of metabolic function in the embryonic cells of the heterotic F_1 hybrid, we applied [U-^{14}C] glucose to the kernels at each germinating stage, and evaluated incorporation rate of radioactivities into CO_2 and the various cellular components. The experiments were done for the heterotic F_1 hybrid and its parental inbred lines. Results are shown in Table 3.

Since the aleurone cells also contribute to the kernels' respiration, the incorporation rate of ^{14}C-activities into CO_2 were expressed on a whole kernel basis, while the rest of the results were shown on the basis of dry weight of the embryo. Through 12 hrs of imbibition, there are no significant differences between the F_1 hybrid and either parent in terms of the incor-

Table 2. CO_2 evolution rate at different times after imbibition of the kernels.

Time after imbibition (hr)	μl CO_2 evolved hr^{-1} $kernel^{-1}$				
	Oh545	Oh45	W22	Oh545×Oh45	Oh545×W22
4	13.6a*	14.0a	8.8b	10.2a	14.0a
16	28.9b	30.3a	20.4c	22.4c	36.9a
28	43.3b	46.2b	33.2c	35.2c	56.1a
40	61.2b	62.0b	49.9c	51.0c	77.7a
52	87.6b	78.0c	85.1b	80.0c	121.4a

* : Within each row, values followed by the same letter do not differ ($P \geqq 0.05$).
Values represent the average of 3 replicates.

poration rate into each fraction. The time course of the incorporation of radioactivities into the CO_2 fraction was in parallel with the increase in the evolution rate of CO_2 (Table 2 and 3). This suggest that the F_1 hybrid consumed more glucose as a substrate for respiratory metabolism than the parents at the later stage of germination. In order to clarify whether the F_1 hybrid also assimilated more glucose to the primary metabolites in the cell, the radioactivities incorporated into amino acids, organic acids, nucleic acids and proteins were analyzed. For all components except sugar, the F_1 hybrid showed the largest values after 24 hrs of imbibition among the lines examined.

Two possible explanations can be envisaged for generation of the different rate of ^{14}C incorporation into CO_2 and each cellular component among the lines. The first explanation is that uptake rate of [U-^{14}C] glucose into kernels is different among the lines. The second explanation is that assimilation rate of [U-^{14}C] glucose is different among the lines. In the present study, the radioactivities in sugar fraction at each germinating time are generally higher than those in other cellular components, e.g. the values at 48th hr are 30 to 60 times lager than those of organic acids fraction among the lines. Furthermore, the radioactivities in sugar fracrion between F_1 hybrid and the parental lines are similar during whole germinating time examined. These results ruled out the first explanation. Thus, quick assimilation of glucose into each metabolite indicates that the F_1 hybrid utilizes glucose more actively in the generation of energy and metabolites. The residual fraction is mostly composed of cellulose, hemi-cellulose and/or other polysaccharides in the cell wall fraction. More than 10-fold increase of the incorporation into this fraction between the 12th and 24th hr of germination suggests that the cell wall synthesis began in the embryo tissue during this stage. The increase in embryo dry weight after 24 hrs serves to explain this phenomenon. Taken together, these results indicate that the vigorous seedling growth of the heterotic F_1 hybrid appears to be due to the higher metabolic function of glucose in the embryo cells as compared with the parental inbred lines.

Discussion

We have previously shown that the heterotic F_1 hybrid Oh545×W22 germinated faster than its parental lines ; radicle emergence rate at 48th hr of imbibition was 70% and 50% for heterotic F_1 hybrid and parental lines, respectively[11]. However, this early appearance of hybrid vigor was not seen in non-heterotic F_1 hybrid, Oh545×Oh45[11]. In the present study, we have compared the rates of seedling growth and CO_2 evolution from kernels among the heterotic F_1 hybrid (Oh545×W22), the non-heterotic F_1 hybrid (Oh545×Oh45) and their parental inbred lines (Tables 1 and 2). Heterotic and non-heterotic nature of respective F_1 hybrid was also observed in seedling growth rate and CO_2 evolution rate. In our previous studies[9~13], we demonstrated that vigorous syntheses of nucleic acids and proteins in the F_1 hybrid embryo were related to the appearance of hybrid vigor during germination. McDaniel[8] stated that an advantage in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in the cells of the F_1 hybrid contributes a capability for more active cell division and a higher growth rate. For more comprehensive analysis of the

Table 3. Incorporation rate of radioactivities into CO_2 and cellular components in embryo tissue of germinating maize kernels.

Cellular components in embryos	Time after imbibition (hr)	Radioactivity (dpm hr^{-1} gr^{-1}, DW. embryo)		
		Oh545	W22	Oh545×W22
CO_2**	6	27a*	28a	27a
	12	857ab	850b	1,212a
	24	7,457b	5,804c	11,654a
	36	21,740b	14,975c	49,784a
	48	36,440b	39,572b	70,869a
Amino acids	6	2,283a	1,407a	2,027a
	12	3,448a	2,135b	3,869a
	24	7,530b	4,946c	16,412a
	36	22,843b	11,962c	36,465a
	48	53,702b	34,765c	82,208a
Organic acids	6	1,716a	1,500a	2,164a
	12	4,051ab	3,155b	5,000a
	24	11,242a	7,464b	12,352a
	36	13,386b	10,870b	36,954a
	48	17,916b	17,187b	41,667a
Nucleic acids	6	8,183a	2,278b	7,388a
	12	12,931a	6,263b	12,850a
	24	70,823b	42,250c	92,471a
	36	147,875b	143,925b	230,043a
	48	626,089ab	569,406b	707,680a
Protein	6	499a	499a	607a
	12	1,862ab	1,245b	2,050a
	24	61,351b	55,357c	107,691a
	36	134,952b	136,877b	163,592a
	48	542,275b	496,313b	663,486a
Residue	6	183a	240a	171a
	12	344a	463a	340a
	24	5,124b	5,357b	9,853a
	36	15,405ab	10,068b	16,523a
	48	42,965ab	32,703b	58,597a
Sugar	6	14,450a	8,704a	14,604a
	12	26,034a	22,704a	24,599a
	24	246,376a	225,006a	271,915a
	36	424,444a	398,629a	433,482a
	48	1,159,615a	882,194a	1,188,753a

* : Within each row, values followed by the same letter do not differ ($P \leq 0.05$). Values represent the average of 3 replicates

** : The rate of $^{14}CO_2$ evolution is expressed on the basis of a single kernel (dpm hr^{-1} $kernel^{-1}$).

metabolic function, however, consumption of glucose in the cells must also be considered, since this process not only generates energy but also provides various metabolites indispensable to cell growth[2].

To examine this more closely, *in vivo* labelling experiments with [U-^{14}C] glucose were conducted for heterotic F_1 hybrid and its parental inbred lines. The assimilation of glucose to the cellular components is more active in the F_1 hybrid than in the parental lines after 24 hrs of imbibition (Table 3). The similar radioactivities in the sugar fraction between F_1 hybrid and the parents indicated that the uptake of [U-^{14}C] glucose into embryo cell is not a limiting step for the generation of different rates of incorporation into each cellular component among the lines. Thus, we concluded that F_1 hybrid assimilated glucose more actively than did the parental lines. Glucose is mainly assimilated into each cellular components through glycolytic pathway and tricarboxylic acid (TCA) cycle. In early events of seed germination, the integrated function of these metabolic pathways in the cell is important for efficient generation of energy and other indispensable components[3,7,15]. These observations suggest that inefficient assimilation of glucose through these metabolic pathway to organic acids, amino acids and other metabolites decrease the cell's biochemical activities to low level and this circumstance in the embryo cells of the seeds induce slower germination after imbibition. Our previous study indicated that the F_1 hybrid starts to degrade the storage protein in the embryo earlier than do the parental lines[12]. This quick generation of the nitrogen would be favorable for active synthesis of amino acids and nucleotides in connection with a high activity of glucose assimilation in the F_1 hybrid. These biochemical advantages in F_1 hybrid cells lead more active *de novo* synthesis of nucleic acids and proteins. Since we did not measure the incorporation of radioactivity into RNA and DNA separately, the contribution of glucose assimilation to these fraction is not clear from the present experiment. But our previous studies, using ^{3}H-thymidine and ^{3}H-uridine, indicated that the heterotic F_1 hybrid embryos synthesized RNA, and not DNA, much more than the parents after imbibition[10,13]. A high level of radioactivity in the residual fraction indicates that the active cell wall construction of the F_1 hybrid embryo is also sustained by higher metabolic function of glucose. An elevated glucose assimilation activity in the cell could be controlled by higher enzyme activities concerning to glucose metabolism. However, we could not see hybrid vigor manifestation for the activities of aldolase (enzyme of glycolysis) and mitochondrial malate dehydrogenase (enzyme of TCA cycle) in heterotic F_1 hybrid compared with its parental inbred lines during germinating period (unpublished data). This contradictory results could be due to that these two enzymes do not play a role for rate limiting regulation of glucose assimilation. Hageman et al.[4] reported that, even though enzymatic activities does not show any vigor, better balanced metabolic function in the F_1 hybrid cell provide more final products in each metabolic pathway and this biochemical circumstance induce the hybrid vigor appearance. The result of [U-^{14}C] glucose feeding experiments indicated that heterotic F_1 hybrid raised metabolic function of the cell more quickly than the parents after water uptake into embryo and kept them high level throughout the germination period. These advantages observed in metabolic processes seem to be closely related to the vigorous growth rate of the F_1 hybrid embryo (Table 1).

In conclusion, we showed here that quick response to raise the metabolic function in the maize heterotic F_1 hybrid's embryo cell after water uptake was one of the key factors for hybrid vigor manifestation. Furthermore, heterozygotic nature of heterotic F_1 hybrid embryo resulted better balanced or no neck metabolic processes throughout germination. On the other hand, slower response to raise the metabolic function after water uptake and metabolic neck present in the parental inbred lines showed the reduced germination rate. The genetic mechanism which induce the efficient response to the environmental cue should be investigated in order to understand the hybrid vigor throughout the plant's entire life cycle.

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