Responses of Soybeans to Water Stress During Germination

Ⅲ. Respiration of Soybean Seeds During Imbibition

Kim, Y. W.* and H. C. Minor**

土壤水分條件에 따른 大豆의 發芽反應에 관한 硏究

第3報 浸種時間에 따른 大豆種子의 呼吸率 變異

金容旭* · 해리 마이너**

ABSTRACT

Respiration of whole seeds and seed parts isolated from four soybean varieties Essex, Pickett, Wayne and Bonus as measured at 25°C during germination.

The average respiration rates of cotyledons were significantly higher than those of other parts. The values were 35.7, 28.0 and 23.9 ul hr^{-1} $seed^{-1}$ for cotyledons, embyros, and hila, respectively. Bonus showed a significantly lower respiration rate than the other varieties did. The interaction of variety x seed part was not significant source of variation for respiration. This suggests that seed part and variety independently contribute to soybean seed respiration during early imbibition.

The effects of imbibition time and time x variety intereaction on whole seed respiration were significant, but the variety effect was not significant. The correlation coefficient between seed moisture content and respiration after 24 hours of imbibition was significant for Bonus, but not for the other varieties tested. This suggests that Bonus may require more water for respiration during imbibition than the other varieties. There as no significant correlation of whole seed respiration rate after 24 hours imbibition with seed weight or seedling length.

INTRODUCTION

Seed respiration involves the oxidative breakdown of organic components to provide biological energy (ATP) which is utilized for active germination and seedling growth. Much efforts have been devoted to determining seed and seedling vigor. Respiration has been focused on as criterion for studying seed vogor.

In been (*Phasaulus valgaris* L.) seed, germination is accompanied by a marked increase in respiratory activity.[13] A large increase in ATP occurs within an hour on wetting seeds of radish (*Raphanus sativus* L.) and remains constant until the radicle emerges. Respiration of radish seeds accelerates during the first phase of water uptake as described by Moreland *et al.*[10] In the second phase, be-

*Crop Experiment Station, Office of Rural Development, Suweon, Korea 170, ** Agronomy Department, University of Columbia－Missouri, Columbia, Missoui, U. S. A.

* 作物試驗場, ** 美國미조리州立大學校 農科大學

tween 1.5 and 8 hours, the rate of respiration does not appreciably change, but a gradual increase in rate of respiration occurs between 8 and 16 hours.[10]

Oxygen absorption has been used as an index of seed vigor.[6,7,17,18] Ching[6,7] indicated that there is a relationship between ATP level in an imbibed seed and seedling vigor. In crimson clover (*Trifolium incarnatum* cv. Dixie), the ATP content of seeds imbibed for 4 hours is correlated with seed size, weight and length of developing seedlings.[7] Burris[5] indicated that oxygen uptake per seed was greater in large soybean seeds. The overall higher respiratory rate on a seed basis by large seeds may have been due to a higher initial cell number or higher cell division rate. Small seeds had a higher initial respiration rate on a weight basis, possibly because surface area/seed weight decreases with an increase in seed weight.[5]

Development of mitochondria in storage tissues of seeds has been of interest because of their potential role in the modification and utilization of these tissues during imbibition. In barley seeds, an increased quantity of mitochondrial protein in seedlings from heavy seeds is indicative of a higher respiratory rate and greater amount of ATP production.[9]

Some development of mitochondria in pea seeds occurs within the first days after imbition.[3] The existence of two phases in development of mitochondria in the seed during imbibition has been proposed.[11] In phase one, enzymatic activities in the mitochondria increase rapidly. Increase in respiratory activity, protein and lipid contents follow in the second phase. Nawa and Asahi[12] have shown that the early development of mitochondria activity in imbibing pea cotyledons occurs as a result of incorporation of preexisting cytoplasmic protein into immature mitochondria. In a previous study[11], they found that cytochrome oxidase and malate dehydrogenase activity in the seeds mitochondrial fraction increased during the initial phase of imbibition.

Aging enhances seed deterioration. Respiration becomes progressively weaker as deteriroation proceeds and germination is lost. However, prior to loss of germinability of corn, respiration level during the early stages of germination is closely correlated with subsequent seedling vigor[17]. The aging process has been suggested to involve degradation of the respiratory system[5], loss of ATP production capacity[7] and degradation of membranes[16]. Changes in respiratory activity following accelerated aging has been shown in soybean seeds. The consumption of O_2 declined with increasing time of aging and respiratory rate was less than half of that of the control after 7 days of aging[14].

Severe dehydration leads to a pronounced decrease in seed respiration causing drought injury4). Hegarty[8] reported an interaction between germination, water uptake and O2 uptake of carrot (*Daucus carota*) seeds under moisture stress. The water content and oxygen uptake rate in -5 bars osmotic solution was lower than in water. At -15 and -20 bars, water content remained constant through the experiment but oxygen uptake fell after 6 days. Ashraf and Abu-Shakra[2] observed that there were no significant differences in seed respiration rates among wheat cultivars during the initial 12 hours of imbibition in 0, -9, and -15 bars osmotic solutions, but differences became pronounced with increases in moisture stress and at advanced stages of germination. Respiration rates were found to decrease with increase of moisture stress probably due to lack of water uptake by seeds[2]. The early period of germination has been shown to require a normal O_2 tension, but this is no longer a requirement at later stages. An alternate pathway of respiration provides for the completion of the earlier stage of soybean germination[15].

The objective of this study was to evaluate the usefulness of respiration as an indicator of soybean seed germination under water stress.

MATERIALS AND METHODS

This study employed the medium seeded size from each variety; Essex, Pickett, Bonus and Wayne. Ten seeds were imbibed in a petri dish lined with two layers of filter paper (Whatman No. 1) wetted with 10 ml of distilled water at 25°C. At four hours intervals for 24 hours, two seeds per replication were transferred from petri dishes to a 17 ml Gilson respirometer flask containing 1 ml of deionized water. In addition, dry seeds were carefully separated with a razor blade into an embyronic axis, cotyledons, and hilum containing a small fraction of seedcoat. The seed parts were then imbibed in a petri dish at 25°C for one hour. After imbibition, two samples per replication of each seed part were transferred to a respirometer flask containing 1 ml of deionized water.

A CO_2 free atmosphere was maintained by absorption of CO_2 in 0.2 ml of 20% KOH (W/V) placed on a filter wick in the center well of the flasks. The flasks were equilibrated in a water bath at 25°C. After respiration measured, the two intact seeds were removed from each flask and moisture content was determined. Moisture contents were measured by drying at 95°C for 24 hours. The whole seed respiration (not seperated) and seed part respiration were consisted of 3 and 4 replications, respectively.

All data were subjected to analysis of variance. The 5% level of probability was applied to compare treatment means. Correlation coefficients between seed weight, seed moisture content, seedling length and respiration of whole seed after 24 hours imbibition were computed for each variety.

RESULTS AND DISCUSSION

1. Respiration of Seed Parts

Respiration rates of seed parts (cotyledons, embyros and hila) isolated from soybean seeds of four varieties were measured at 25°C after one hour imbibition.

The effects of variety and seed part on the respiration rates of soybean seeds were highly significant. The interaction of variety x seed part was not significant. Reduction of seed vigor has been reported to be associated with a decline in respiration and in protein and carbohydrate synthesis, and with an increase in permeability of membranes of embyronic exes[1]. Development of mitochondria in storage tissues of seed has been of interest because of their potential role in modification of the stored reserve materials during imbibition. Opik and Simon[13] indicated that germination of a seed is accompanied by a rapid water uptake rate and marked rise in respiration, and that this rise in respiration rate is due to the activation enzymes already present in dry seed and to the growth and development of the seed tissues.

The respiration rates of cotyledons were significantly higher than those of embyros and hila following one hour of imbibition, but there were no significant differences in the respiration rates of embyros and hila (Table 1). The average respiration rates were 35.7 for cotyledons, 28.9 for embyros, and 23.9 ul hr^{-1} $seed^{-1}$ for hila. These results suggest that the storage organs are more active in seed respiration during early imbibition than are other organs. However, the weight of cotyledons are heavier than those of embyros and hila. Thus, the respiration rates of embyros on a gram weight basis are higher than those of cotyledons. The hilum acts as a pathway for gas exchange between the embyro and external environment. Thus, the structure of hilum may have an effect on metabolism. It is possible that the metabolic changes in seed during imbibition may be retarded if gas exchange through the hilum region is prevented. In general, cotyledons are most sensitive to adverse environmental conditions during periods of storage due to metabolic changes of reserve materials. Thus, seed vigor may be closely related to the quality of cotyledons. For example, Abdu-Baki and Anderson[1] indicate that loss of seed vigor may be due to impairment of biochemical or physiological functions, and that the types of metabolic changes are specific and dependent on the nature of factors that induce a particular change.

The respiration rates of Essex, Pickett and Wayne were significantly higher than those of Bonus

Table 1. Mean respiration rates of seed parts of four soybean varieties after one hour imbibition.

Variety	Seed Part			Average for variety
	Cotyledon	Embyro	Hilum	
	O_2 uptake $\mu l\ hr^{-1}\ seed^{-1}$			
Bonus	25.4	20.8	14.0	20.1
Wayne	32.6	29.8	28.4	30.3
Essex	44.8	29.3	24.5	32.9
Pickett	39.8	32.2	28.7	33.6
Average for part	35.7	28.0	23.9	29.2

LSD .05 = 4.6 for average for variety.
= 5.3 for average for part.

(Table 1). There were no significant differences in the seed respiration rates among Essex, Pickett and Wayne following one hour of imbibition. In this study, the low respiration of Bonus may have been related to low initial seed quality which may be closely associated with quality of cotyledons.

2. Respiration of Whole Seed

Respiration of whole intact seed was measured after 4, 8, 12, 16, and 24 hours imbibition at 25°C. The effects of time and time x variety interaction on the respiration were highly significant. The respiration rates were significantly increased with increase in imbibition time, but not significant between 8 and 12 hours imbibition. The average respiration rates ranged from 13.6 ul hr^{-1} $seed^{-1}$ after 4 hours imbibition to 25.3 after 24 hours imbibition (Table 2).

The pattern of seed respiration has been reported by Opik and Simon[13] and Moreland *et al*[10]. Opik and Simon[13] classified three phases of seed respiration during germination. The first phase is closely dependent on water content of cotyledons. The second phase is characterized by a 'pause' in both water uptake and respiration rates. The fact that the respiration rate may decrease during this period raised the possibility of a temporary shortage of available substrate from the seed. The respiration and water uptake rates rise during the third phase since metabolic activity is rising. A study of Moreland *et al*[10] with radish seed indicated that oxygen uptake by unimbibed

Table 2. Mean respiration rates of whole seed of four soybean varieties at different imbibition times.

Variety	Imbibition (hours)					Average for variety
	4	8	12	16	24	
	O_2 uptake hr^{-1} $seed^{-1}$					
Bonus	10.3	16.6	18.0	24.4	22.9	18.4
Wane	13.9	18.3	16.2	23.3	24.4	19.2
Essex	15.2	14.1	17.3	24.9	26.3	19.6
Pickett	15.0	16.1	19.6	20.4	27.8	19.8
Average for time	13.6	16.3	17.8	23.2	25.3	19.3

LSD .05 = 1.5 for average for time.
= 3.4 for interaction of time x variety.

seed was too law to be detected, and that respiration was initiated during water uptake. In the second phase, between 1.5 and 8 hours, the rate of respiration did not change. Between 8 and 16 hours imbibition, the seed respiration rate increased due to emergence of the radicle. In the present study, the rapid increase in the respiration rates of soybean seeds occurred during the first 4 hours imbibition. Between 4 and 24 hours, the respiration continued to increase linearly (Figure 1).

The average respiration rates were 18.4 ul hr^{-1} $seed^{-1}$ for Bonus, 19.2 for Wayne, 19.6 for Essex, and 19.8 for Pickett (Table 2). These results are in agreement with the findings of Ashraf and Abu-Shakra[2)] who indicated that there were no significant differences in seed respiration among wheat cultivars during the initial 12 hours of imbibition. They found that differences became

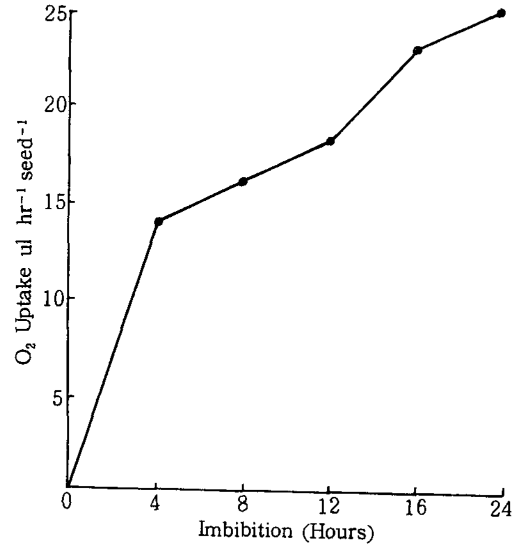

Fig. 1. Respiration rate of soybean seeds during imbibition.

Table 3. Correlation coefficients between seed weight, seed moisture content, seedling length and respiration after 24 hours imbibition.

Variety	Characters	Seed weight (g)	Seed moisture (%)	Respiration (μl hr^{-1} $seed^{-1}$)
Bonus	Seed weight			
	Seed moisture	-0.05		
	Respiration	-0.06	0.64**	
	Seedling length (cm)	0.08	0.03	-0.009
Wayne	Seed weight			
	Seed moisture	-0.09		
	Respiration	0.03	0.21	
	Seedling length (cm)	-0.10	0.02	0.05
Essex	Seed weight			
	Seed moisture	-0.23		
	Respiration	-0.20	0.08	
	Seedling length (cm)	0.02	0.10	0.05
Pickett	Seed weight			
	Seed moisture	-0.18		
	Respiration	-0.03	0.10	
	Seedling length (cm)	-0.37	0.14	0.10

** Significant at the 1% level.

Seed moisture notes seed moisture content during 24 hours imbibition.

Seedling length measured 3 days after planting in petri dish.

pronounced with an increase in moisture stress, probably due to lack of water uptake by seeds. In this study, the correlation coefficients between seed moisture content and respiration rate for the four varieties tested were not consistently significant. The coefficient for Bonus was highly significant (r=.64). The coefficient for Wayne was *higher than those of Essex and Pickett, but was not* significant (Table 3). A possible explanation is that Bonus and Wayne may have lower respiration than do Essex and Pickett under water stress because of differences in water uptake of the seeds.

A higher cell number has been shown to be related to an overall higher respiration rate per seed in large seeds. In barley seed, an increased quantity of mitochondrial protein in seedlings from heavy seeds is indicative of a higher respiration rate and greater amount of ATP production[9]. In this study, the correlation coefficients between individual seed weight and respiration rate after 24 hours imbibition were not significant (Table 3). The correlation coefficients between 3 day seedling lengths and respiration rate after 24 hours imbibition were nbt significant for any variety tested in this study (Table 3). These results are not in agreement with findings of Woodstock[17] and Woodstock and Grabe[18] who found a significnat positive correlation between seedling growth and respiration during imbibition.

摘　要

本 試驗은 大豆 種子의 呼吸率의 品種間 差 및 種子 部位別 (子葉, 胚軸, 배꼽) 差를 檢討하고, 이러한 呼吸率의 差가 發芽에 미치는 影響을 究明함으로서 效率的인 高發芽性 種子의 初期 選拔을 위한 基礎的 資料를 얻고저 遂行되었다.

1. 1時間 浸種後 大豆 種子의 呼吸率은 種子部位別 및 品種間 差를 인정할 수 있었으나, 種子部位와 品種間 相互作用은 有意性이 없었다. 그러므로 種子部位와 品種은 大豆 種子의 呼吸에 獨立的으로 作用하였다.

2. 子葉의 呼吸率은 다른 部位, 즉 胚軸이나 배꼽에 비해 크게 높았으며 그 平均값은 子葉이 35. 7, 胚軸이 28.0, 그리고 배꼽이 23.9 μl hr^{-1} $seed^{-1}$이었다. 그러므로 大豆 種子의 貯藏器管이 全體的으로 呼吸에 主要한 影響을 미친다고 할 수 있다. 品種 Bonus는 다른 品種들에 비해 낮은 呼吸率을 보였다.

3. 全體 大豆 種子의 呼吸率에 있어서 浸種時間 및 時間과 品種間의 相互作用은 有意性이 인정되었으나 品種間에는 有意的인 差가 없었다. 4時間 浸種 동안에 急激한 呼吸率의 增加를 보였으며, 平均 呼吸率은 4時間에 13.6, 8時間에서 16.3, 12時間에서 17.8, 16時間에서 23.2, 그리고 24時間에 25.3 μl hr^{-1} $seed^{-1}$이었다.

4. 品種 Bonus에 있어서 種子 水分含量과 24時間 浸種동안의 種子의 呼吸率 間에는 有意的인 相關이 인정되었으나, 다른 品種들에 있어서는 相關係數의 有意性이 없었다. 이 結果는 品種 Bonus는 다른 品種에 비해 24時間 浸種동안 呼吸에 보다 많은 水分을 要求한다고 생각한다.

5. 種子무게와 呼吸率 間의 相關係數는 有意性이 없었으며, 이 結果는 大豆 種子의 呼吸率은 種子의 무게와 直接的인 關聯이 없는 것으로 思慮된다. 또한 呼吸率과 3日間의 初期 草長의 伸張間에도 有意的인 相關이 없었다.

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