Response of Sesame (*Sesamum indicum* L.) to Low Oxygen Concentration during Germination

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Abstract: Screening of crop varieties tolerant to a low oxygen environment caused by heavy rain has become an important work in monsoon Asia countries in recent years. We examined the growth of sesame (*Sesamum indicum* L.) germinated for 5 d in air with 0.05 O$_2$ m$^{-3}$ (low oxygen concentration, LO), in comparison with those germinated in air containing 0.20 O$_2$ m$^{-3}$ (ambient oxygen concentration, AO). The growth of sesame was not suppressed but rather accelerated by LO. Immediately after the exposure to LO, seedlings had a 2 fold larger number of secondary roots, and more than 13.0% and 7.4% heavier in leaves and roots, respectively, over those under AO. After the oxygen treatments were over, the seedlings were immediately transferred to normal conditions to grow. One month later, the seedlings exposed to LO accelerated about two-fold dry matter (DM) over those under AO. In spite of lower content of chlorophyll, the leaf area ratio (LAR) and the net assimilation rate (NAR) of the plants exposed to LO were higher than those under AO. Compared to plants under AO, the ultimate leaf size of the cotyledon, the 1st leaf and the 2nd leaf of plants exposed to LO were 20.0, 22.9, and 27.0% greater, respectively. In comparison with pea (*Pisum sativum* L.), sesame respired in a different way. The total biomass yield and grain yield of plants exposed to LO were higher by 8.3% and 11.6% respectively than those under AO. These observations revealed that the hypoxic stress induced some different metabolic processes in the earlier growth stage of the plant and obviously had an advantageous effect on the subsequent growth of the plant.

Key words: Germination, Hypoxic stress, Sesame, *Sesamum indicum* L.

Heavy rain in the summer in the monsoon district of Asia countries is a big problem hindering the normal growth of some crops. The excessive water from the rain makes the soil water-saturated for quite a long time, and it is believed that the excessive water harms the crops by squeezing out the oxygen in the soil, which is necessary for the plant roots to respire. Screening the crop varieties tolerant to a low oxygen environment is now an important subject in Southeast Asia countries.

Higher plants respire and obtain the energy preserved in the respiratory substrate with the expenditure of oxygen to achieve the most effective energy transformation. However, exposure to oxygen deficits is brought out more often in the biological system than has been commonly believed. Until quite recently the prevailing view of anaerobic metabolism was basically synonymous with glycolysis and alcohol fermentation. Consequently, research on the quantitative and physiological effects of hypoxia or anoxia on crop germination has recently begun. Earlier research findings indicated that as oxygen volume decreased from 0.21 m$^3$ to zero, most of the dicotyledonous plants and forage grasses increased the photosynthetic rate because the photo-respiration was suppressed, and that oxygen concentration was not so much associated with the dark respiration (Akita, 1980). A few plants were found to be tolerant to hypoxia/anoxia, including *Zizania tesa* (Texas wildrice) (Hook and Crawford, 1978; Power, 1990), *Erythina caffra* (coral tree) (Small et al., 1989), and *Echinochloa crus-pavonis* (barnyard grass) (Fox et al., 1990; Mujer et al., 1993; Zhang et al., 1994). Some constitutive and inducible anaerobic stress proteins were found and anoxia-induced gene expression was also studied recently (Kennedy et al., 1992; Sachs and Ho, 1986). In a study (Al-ani et al., 1985) on the effect of partial pressure of oxygen on the germination and respiration of 12 cultivated species, seeds of plants were classified into two groups according to their irresponsiveness to low O$_2$ pressure. It was discovered in Japan that rice seedlings emerged better by coating oxygen-generating chemicals on the surface of rice seeds (Nakajima et al., 1996; Osada, 1982). Studies on the method of screening varieties tolerant to hypoxia conditions and some studies in cytochromes in response to air-adaptation have also been in progress (limura et al., 1995, 1996; Shibasaka et al., 1994). On the other hand, most of the relevant studies on hypoxia/anoxia were limited to the measurement of only short-term effects. There were very a few reports on the effect of low O$_2$ conditions during germination on the growth and yield of crops (Arihara and

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Abbreviations: AO, ambient oxygen concentration; DM, dry matter; LAR, leaf area ratio; LO, low oxygen concentration; NAR, net assimilation rate; RGR, relative growth rate.
in each pot were prepared both for exposure to AO and LO. The pots with plants were placed in a greenhouse under natural light conditions and controlled day/night air temperature of 28°C/22°C.

3. Measurements
The dark respiration rate was measured under dark conditions by a method described by Tanaka (1985). It was measured both for sesame and pea plants at 48 hr after the start of O₂ treatment, at and 24 hr, 5 d and 15 d after the end of the oxygen treatment, respectively. The measurement was done at 24–26°C with three replicates.

The growth analysis method was used to assess the effect of LO on the earlier growth of sesame seedlings. Seedlings were sampled once every week for five weeks after transplanting. Seedlings from four pots (20 plants) were sampled randomly each time both for AO and LO. After sampling, leaves, stems and roots were separated and killed at 100°C for 5 min and then dried at 80°C for 48 hr. Finally, the DM was weighed. The leaf area was measured at the same time with an ACC-400 automatic leaf area meter (Hayashi Denko Ltd., Tokyo, Japan). The parameters were calculated as follows:

\[
RGR = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1}
\]

\[
NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{\ln(F_2) - \ln(F_1)}{(F_2 - F_1)}
\]

\[
LAR = \frac{\ln(W_2) - \ln(W_1)}{(W_2 - W_1) \times (F_2 - F_1)} \times \ln(F_2) - \ln(F_1)
\]

where \(W_1\) and \(W_2\) were the DM weights of the crop population at time \(T_1\) and \(T_2\), respectively and \(F_1\) and \(F_2\) were the leaf areas of the population at \(T_1\) and \(T_2\), respectively.

After the O₂ treatment, seedlings were sampled and the characteristics of the seedlings were measured. When the sesame plants for yield structure test matured enough, they were sampled, and analyzed for yield structure. The chlorophyll content was measured with a portable chlorophyll meter (SPAD-502, Minolta, Japan). The infection of sesame plants with the Phoma wilt disease (Phoma exiguum S. f. saxirheum A.) was simply calculated by counting the plants that died from the disease in each pot from transplanting to one month later. All of the difference between the two treatments, when necessary, was analyzed by Student’s t-test method.

Results and Discussion

1. Morphological responses
Soon after the O₂ treatment was completed, seedlings under AO were taller, nearer in dry matter of stems, and longer in taproots than those exposed to LO. By contrast, seedlings exposed to LO had a larger number of secondary roots (more than twice), and heavier dry matter of leaves and roots than those under AO (Table 1). As a result, the ultimate size of the cotyledons, the first and second primary leaves in the plants exposed to LO was 20.0, 22.9 and 27.0% larger than those under
Table 1. Seedling characteristics of sesame determined immediately after the treatments exposing to various oxygen concentrations during germination.

| Treatment | Leaf length | Leaf width | Plant height | Tap root length | Secondary roots | Leaf weight | Stem weight | Root weight | Total weight |
|-----------|-------------|------------|--------------|----------------|-----------------|-------------|-------------|-------------|--------------|
| AO (n=50) | 7.60        | 6.98       | 4.22         | 5.59           | 4.10            | 1.77        | 0.92        | 0.50        | 1.19         |
| LO (n=50) | 7.60        | 7.10       | 2.40         | 4.15           | 8.80            | 2.00        | 0.62        | 0.54        | 3.16         |

*Significantly different at the 0.05, 0.01 and 0.001 probability levels, respectively.

Samples were sampled immediately after the 5-day oxygen treatment. All the leaves on the plant were measured.

AO, respectively, and the differences were significant (Fig. 1). The percentage of dry matter allotted to roots and leaves during the first month after transplanting was somewhat higher in the plants exposed to LO than in those under AO (Table 2). Thus sesame exposed to LO possessed more DM in leaves and roots than that under AO, which would be a better structure for further growth.

Table 2. Partitioning with time of DM accumulation (expressed as %) exposed to various oxygen concentrations for 5 days during germination.

| Time course | Leaves | Stems | Roots |
|-------------|--------|-------|-------|
|             | AO     | LO    | AO    | LO    | AO    | LO    |
| 1st week    | 55.5±5.6 | 63.3±5.9 | 28.8±3.0 | 19.6±2.5 | 15.7±2.0 | 17.1±1.3 |
| 2nd week    | 48.8±5.2 | 54.1±5.5 | 31.4±3.0 | 20.7±2.4 | 19.8±2.0 | 25.2±2.0 |
| 3rd week    | 56.9±5.5 | 58.0±5.8 | 22.1±2.5 | 15.5±2.0 | 21.0±2.5 | 26.5±2.2 |
| 4th week    | 61.1±5.8 | 61.1±6.3 | 21.5±2.4 | 19.6±2.1 | 17.4±2.2 | 19.3±1.9 |
| 5th week    | 60.6±5.8 | 58.1±6.2 | 25.5±2.8 | 25.6±3.0 | 13.9±2.0 | 16.3±1.2 |

Seedlings were transplanted at the end of the oxygen treatment and DM was measured weekly after transplanting. Data are Mean±SE.

n = 20 for each time and each treatment.
higher in the plants exposure to LO than in the plants under AO during the first two weeks, and the difference was the largest in the first week (Fig. 5). On the other hand, LAR of the plants exposed to LO was about 10% higher than that of the plants under AO during the first four weeks after transplanting (Fig. 5). Thus the plants exposed to LO grew more vigorously than those under AO, although they had a lower chlorophyll content initially. This may be because they had a larger leaf area and accumulated a larger amount of DM in the leaves and roots.

3. Yield response

The total biomass and grain yields of the plants exposed to LO were, respectively, 8.3% and 11.6% higher than those of the plants under AO (Table 3). These differences were significant as indicated in the table. The DM weight of roots and leaves, and yield components was also increased by the exposure to LO (Table 4).

From the present results, we concluded that sesame plants exposed to LO germinated advantageously, resulting in better growth and greater yield than those ger-
Table 3. Biomass yield and grain yield of sesame as affected by low oxygen concentration during germination.

| Treatment | Biomass yield (g plant⁻¹) | Root weight (g plant⁻¹) | Stem weight (g plant⁻¹) | Leaf weight (g plant⁻¹) | Capsule weight (g plant⁻¹) | Grain yield (g plant⁻¹) | Harvest index (%) |
|-----------|--------------------------|-------------------------|-------------------------|-------------------------|----------------------------|------------------------|------------------|
| AO (n=6)  | 50.40±0.40               | 5.01±0.26               | 14.66±0.67              | 8.50±0.10               | 22.23±0.19                 | 12.88±0.62             | 25.56±0.29       |
| LO (n=6)  | 54.96±0.73               | 7.70±0.30               | 14.90±0.17              | 7.70±0.20               | 24.66±0.29                 | 14.57±0.40             | 26.51±0.10       |
| t-test    | **                       | ***                     | ns                      | **                      | ***                        | *                      | *                |

*, **, *** Significantly different at the 0.05, 0.01 and 0.001 probability levels, respectively. Data are Mean±SE.

Table 4. Plant characteristics and yield components of sesame at maturity as affected by low oxygen concentration during germination.

| Treatment | Plant height (cm) | Nodes with capsules | Capsules with grains | Capsules with no grains | Grains per capsule | 1000-grain weight (g) |
|-----------|-------------------|---------------------|----------------------|------------------------|-------------------|-----------------------|
| AO (n=6)  | 162.50±5.52       | 29.50±1.38          | 21.70±3.19           | 81.10±4.29             | 6.30±1.03         | 62.00±5.22            |
| LO (n=6)  | 163.20±5.48       | 33.80±1.05          | 24.50±1.38           | 87.30±1.64             | 4.20±0.75         | 65.10±2.92            |
| t-test    | ns                | **                  | ns                   | ns                     | **                | **                    |

*, ** Significantly different at the 0.05, 0.01 probability levels, respectively. Data are Mean±SE.

minated under AO, and that the effect was observed from a very early stage of growth.

Sesame has been reported to possess a unique property in its response to hypoxic stress in germination differing from all of the other crops such as barley, pea, cotton, rice, and peanut (Tian and Arihara, 1998). Since the distinct morphological and physiological changes were induced by exposure to LO as already discussed, studies on the biochemical process within the plant would be of great interest. Moreover, Dennis et al. (2000) reviewed the recent progresses in identifying genes and gene products of plants induced during LO conditions, and outlined three stages of plants responding to oxygen deficit. All of the three stages were considered to happen within merely 0–48 hr of the LO treatment, and among them, the first stage (0–4 hr), namely the earlier response, was the most critical. We can suppose that in our experiment with sesame, from the initial stage of germination, some alterations in gene expression and the corresponding metabolic pathways were induced by exposure to LO. Further researches in gene expression and metabolism would be necessary and critical (Dennis et al., 2000; Sachs and Ho, 1986). So far almost all of the research on the effect of hypoxic stress on plants has been focused on the relatively short–time responses (transient effect) rather than the influences on morphological development and the final yield of crops. Furthermore, among crops, no acceleration by hypoxia/anoxia, but various resistances/tolerances to them had been reported so far. Therefore, the obvious acceleration in the earlier growth stage and the notable increase in the grain yield by the exposure to LO in sesame have great implications. Furthermore, it was also observed that seedlings exposed to LO were less infected with Phoma wilt disease in the earlier growth stages. The average infection rate in the plants exposed to LO was 5.0% but that in the plants under AO was 9.8%.

It is clear that a low O₂ concentration during germination not only accelerated the growth of sesame in earlier stages, but it might also promote the later growth of the plant.

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