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Temperature and Gibberellin-induced Respiratory Changes in *Capsicum annuum* during Germination at Varying Oxygen Concentrations

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Abstract. **Concentrations of O_2 greater than 21% stimulated germination rate of *Capsicum annuum* L. (sweet pepper) at 25°C but inhibited germination rate at 15°. At a 10% O_2 concentration, germination rates were reduced at both temperatures. Gibberellins A_4A_7 (GA_{4+7}) increased germination rates at 15° and 25° in air. At 25° in 100% O_2, germination rates of GA-treated and nontreated seeds were the same. At 15° and 100% O_2, germination rates were increased slightly by GA_{4+7} application; however, the rates were slower than in air. Total respiratory activity at 25° was higher in 100% O_2 than in air. High O_2 concentrations did not affect the proportion of respiration which was in the cyanide-sensitive and -resistant pathways. Cyanide-resistant respiration comprised only a small percentage of total respiratory activity. At 15°, total respiration and the cyanide-sensitive and -resistant components were similar regardless of O_2 treatment. The addition of GA increased respiratory activity only after radicle emergence occurred. Thus, germination rate of pepper seed can be increased by increasing temperature, higher O_2 concentrations at the higher temperature, and GA_{4+7} at normal and low temperatures. GA appears to affect germination through metabolic events which occur before radicle emergence and which do not include alteration of respiratory activity.**

With the exception of seeds of certain aquatic plants, seeds will not germinate without O_2 (3). During early imbibition, respiration rates of rehydrated embryos may increase even though O_2 concentrations are low (3). Water that is covering the structures of the seed during imbibition can act as a barrier to O_2 and the only O_2 which can pass to the embryo must be dissolved in that water.

Increased germination and germination rate of several species has been achieved by increasing the O_2 concentration (4, 6). Sachs et al. (11) found that clay-coated pepper seeds germinated faster in an environment of 100% O_2. They suggested that the clay coating limited O_2 supply to the embryo and thus inhibited germination at O_2 concentrations of 21%.

Oxygen supply regulates respiratory pathways during seed germination (10). In dormant seeds, any treatment that increased alternate respiration tended to decrease or alleviate dormancy. Such treatments included the removal of covering structures, increased O_2 pressure, and application of respiratory inhibitors to the cytochrome-mediated cyanide-sensitive pathway. Higher O_2 pressure may have stimulated alternate respiration because the oxidase of this pathway has a low affinity for O_2. Yentur and Leopold (15) concluded that the alternate respiratory pathway was needed during the early stages of soybean germination. In addition, they found that the early stages of soybean germination required a normal tension of O_2, but later when alternate respiration was lower, this was no longer required. Burguillo and Nicolas (2) reported that the alternate respiratory pathway was not the major pathway early in the germinations of chick peas, but reached its maximum between 72 and 96 hr after imbibition. Oxygen stimulated the appearance of the alternate respiratory pathway, and this stimulation was dependent on cytoplasmic protein synthesis.

Germination rate of bell pepper seeds is greatly reduced on temperature decreases below 18°–20°C. In an attempt to determine if temperature and O_2 could alter respiratory rates and pathways during early germination, cyanide-sensitive and -resistant respiration was studied. GA has been shown to stimulate more rapid germination rates in pepper (14). The relationship of this promotive effect to altered respiratory activity was also determined at different temperatures and O_2 levels.

Materials and Methods

Effect of O_2 on germination. Twenty-five pepper seeds ('Early Calwonder') were placed on a moistened filter paper (Whatman, No. 3) in open 5.5-cm Petri dishes. The Petri dishes were placed in 450-ml jars and sealed. The lid to each jar had inlet and outlet vents fitted with rubber septa. Jars receiving the same gas treatments were connected via needles and rubber tubing. The jars were placed in constant-temperature chambers, 15° or 25°C, and attached to gas streams containing the desired atmospheric concentrations of O_2 and N_2. The gas streams were humidified by bubbling them through distilled water at the desired germination temperature. Oxygen mixtures of 10, 21, 40, 60, and 100% were achieved by mixing 100% O_2 and 100% N_2 in a 1000 ml Erlenmeyer flask, after passing the gases through distilled water. Flow rates for individual gases were monitored by the use of flowmeters. The gas mixture, before and after passing through the sealed jars, was monitored with a Beckman oxygen analyzer. Each treatment was replicated 4 times; the duration of the experiments are noted in Fig. 1 and 2.

Effect of O_2 and GA combinations on germination. Twenty-five seeds were placed on a filter paper in an open 5.5-cm Petri dish. Three ml of GA_{4+7} at 100 or 1000 ppm in distilled water was used to moisten the filter paper. The Petri dishes were then

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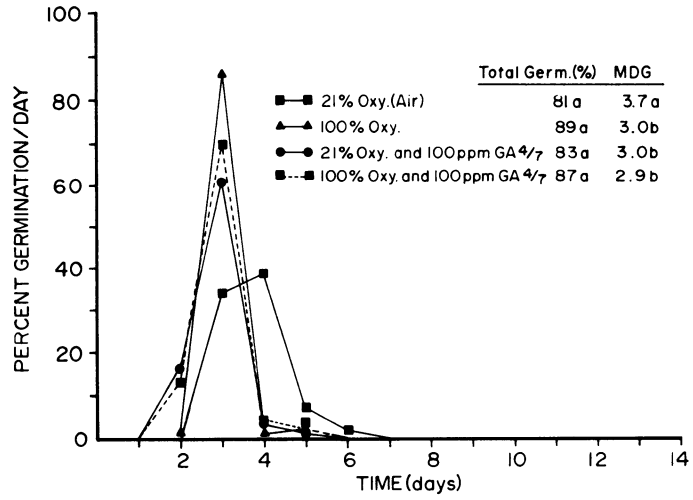

Fig. 1. The effect of GA_{4+7} and O_2 on the germination of pepper seeds at 25°C. MDG = mean number of days to germination. Mean separation within columns by Duncan's multiple range test, 5% level.

placed into 450-ml glass jars. The jars were sealed and supplied with 21 and 100% O_2 gas streams at 15° and 25°C as described above.

Germination data for all the experiments in this section were determined at 24-hr intervals and all treatments were replicated 4 times. Seeds with visible radicles were counted as germinated. Data taken for each treatment included total percent germination and mean number of days to germination (MDG) as previously described (14).

Respiratory measurements. Seeds (100/treatment) were imbibed or germinated in the manner described above in 21 or 100% O_2 at 15° or 25°C for various periods of time. Percent germination was determined and all the seeds were immediately transferred to 15-ml Warburg respirometer flasks. One ml of distilled water was added to each flask with or without KCN (10 mM) or salicylhydroxamic acid (SHAM) at 1 or 10 m M. The center wells contained 0.4 ml of 10% KOH (w/v) and a filter-paper wick to absorb the CO_2. The KOH may have absorbed some CN from the respirometer flask environment (9) but the concentrations of CN in the reaction medium remained sufficient to inhibit maximally O_2 uptake. This was apparent from the observation that any concentration of CN above 5 m M inhibited O_2 uptake to the same extent at both 15° and 25°. Flasks were mounted onto a Gilson differential respirometer, submerged in the water bath, then equilibrated at 15° or 25°. Flasks containing the tissue to be exposed to high O_2 were then flushed via a sidearm on the respirometer flask with 100% O_2 as previously described (13). Flasks were equilibrated for 1 hr before commencing O_2 uptake measurements. Oxygen uptake was measured 3 times for 15 or 30 min with a 15-min equilibration between readings for each treatment. Upon the termination of each experiment, seeds were removed from the flasks, rinsed, dried at 70° for several days, and weighed. Each treatment was replicated 4 times. Results are expressed on a -dry weight basis.

Determination of total respiratory activity in germinating seeds treated with GA_{4+7} at 100 ppm was performed as described above, but without the use of respiratory inhibitors. One hundred seeds were imbibed or germianted in 9-cm Petri dishes in a constant-temperature chamber at 15° or 25°C. Whatman No. 1 and No. 3 filter papers were placed in the bottom of the Petri dish and moistened with 0.05 M pH 6.5 sodium phosphate buffer or 100 ppm GA_{4+7} in buffer solution. Seeds were removed at the appropriate time intervals, total percent germination was determined, and the seeds were placed in Warburg respirometer flasks. One ml of the treatment solution was added to each flask. Oxygen determinations were taken as described above. Treatments were replicated 4 times.

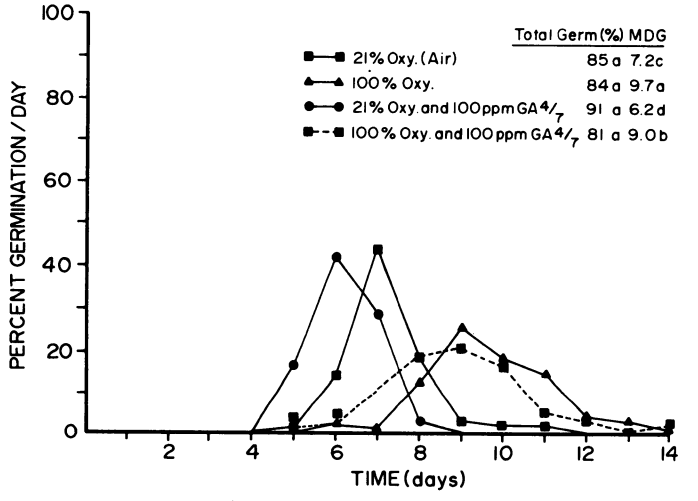

Fig. 2. The effect of GA_{4+7} and O_2 on germination of pepper seeds at 15°C. MDG = mean number of days to germination. Mean separation within columns by Duncan's multiple range test, 5% level.

Results

O_2 above 21% increased pepper seed germination rate at 25°C (Table 1). O_2 at 10% reduced both rate and total germination when compared to the 21% level. No apparent trends in altering total germination were observed by increasing O_2 above 21%, although there was a significant increase at 60% O_2. At 15°, the O_2 concentrations above or below 21% generally reduced the rate of germination. Total germination was unaffected by O_2 treatments at 15°.

At 25°C, GA_{4+7} or 100% O_2 increased germination rate above the air treatment; however, combining both treatments had no further effect on increasing the rate of germination (Fig. 1). Total germination was unaffected by these treatments at 25°. At 15°, all 4 treatments exhibited different germination rates (Fig. 2). O_2 concentrations of 100% delayed germination, while GA_{4+7} in air increased the germination rate above air alone. When

Table 1. The effect of O_2 on germination of pepper seed at 25° and 15°C.

O_2 concn (%)	Germination			
	25°C		15°C	
	Rate (MDG)[z]	Total (%)	Rate (MDG)	Total (%)
10	12.0 a[y]	70.0 c	12.3 a	80.0 a
21 (air)	4.2b	80.3 b	8.0 d	82.7 a
40	3.4 c	86.0 ab	9.0 c	85.0 a
60	3.2 c	88.0 a	8.6 cd	85.0 a
100	3.4 c	80.0 b	11.2 b	81.3 a

[z]Mean number of days to germination.
[y]Mean separation within columns by Duncan's multiple range test, 5% level.

GA_{4+7} was added to seeds maintained in 100% O_2, it increased the germination rate but did not totally overcome the inhibitory effect of 100% O_2. None of the treatments affected total germination at 15°. Increasing the GA_{4+7} concentrations to 1000 ppm did not decrease the inhibition effect of high O_2 on the germination rate of pepper seeds at 15° (data not presented).

Total respiratory activity at 25°C was higher when seeds were germinated in 100% O_2 compared to 21% O_2 (Fig. 3). Cyanide-sensitive respiration was more advice than cyanide-resistant respiration (indicated by the hatched areas in Fig. 3) throughout germination. The alternate respiratory pathway generally accounted for 5 to 8% of the total respiration.

At 15°C, respiratory rates at 21 and 100% O_2 were similar (Fig. 4). However, respiration was higher after 6 or 12 days in 21% O_2. Cyanide-resistant respiration was low and no differences were observed between O_2 treatments throughout germination.

Respiratory activities were similar during the first 2 days of germination at 25°C for GA_{4+7}-treated and untreated seeds (Fig. 5). However, after day 4, GA_{4+7}-treated seeds had higher respiratory activity than untreated seeds. At 15°, GA had no effect on respiration until day 6 when respiration rates were higher in GA-treated seeds. At both temperatures, GA-treated seeds had higher respiration rates than untreated seeds only after radicle emergence occurred.

Discussion

O_2 concentrations above 21% stimulated germination rate at 25°C. At 25°, diffusion of O_2 to the pepper embryo may be limited by solubility of O_2 or covering structures. Increasing the O_2 concentration to 40% or greater may allow the metabolic processes of the embryo to proceed more near optimal rates. This is indicated by the increase in total respiratory activity in 100% O_2 and by the lack of GA_{4+7} stimulation of germination rate in 100% O_2. Ohmura and Howell (8) similarly found that when corn, soybean, and barley tissues were soaked in water at 20°, a marked decrease in O_2 uptake occurred. This was overcome by substituting 100% O_2 for air. They suggested that water reduced O_2 solubility and diffusion through the tissues.

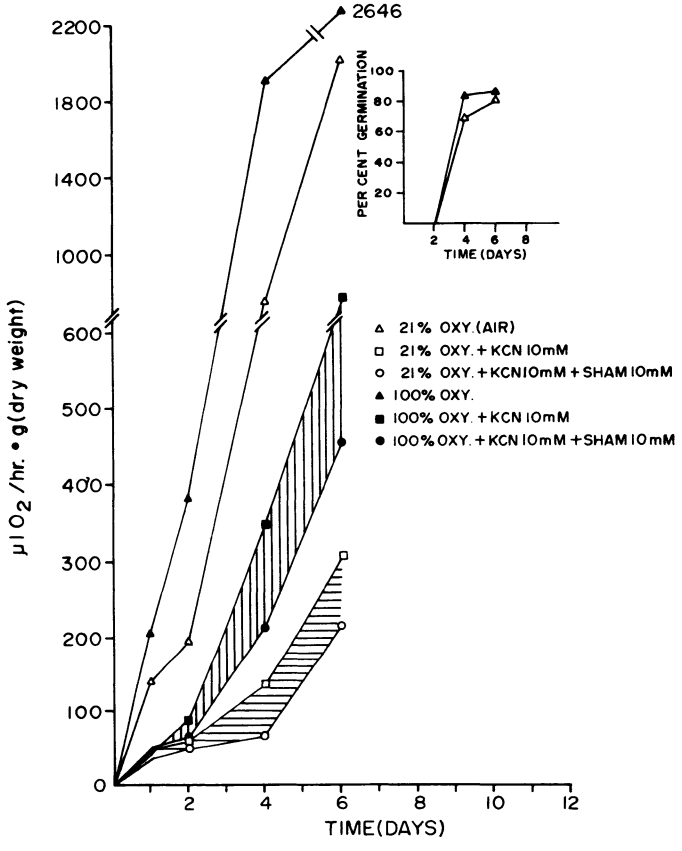

Fig. 3. Respiration (cyanide-sensitive and -resistant components) and percent germination of pepper seeds germinated in 21 or 100% O_2 environments at 25°C.

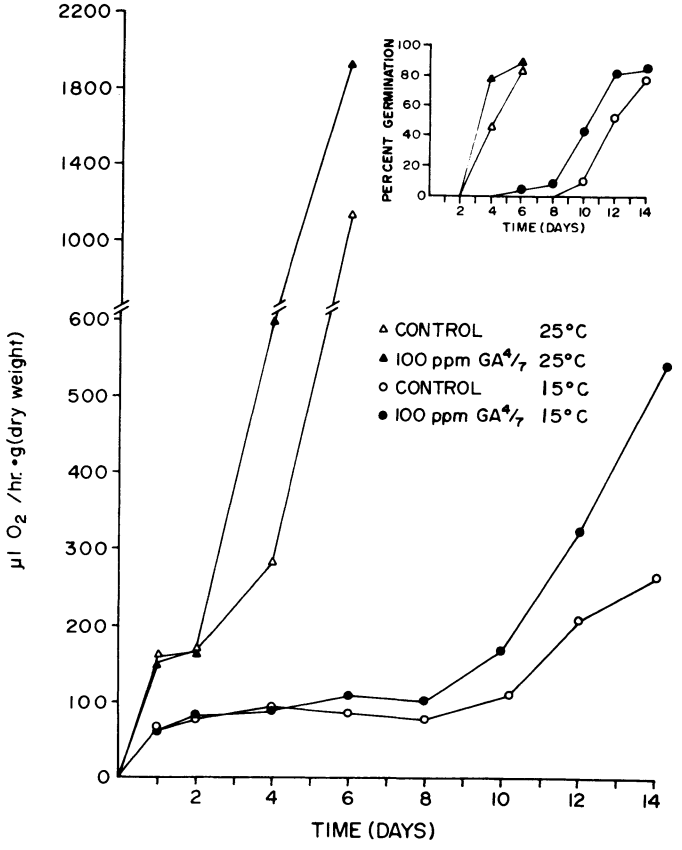

Fig. 4. Respiration (cyanide-sensitive and -resistant components) and percent germination of pepper seeds germinated in 21 or 100% O_2 environments at 15°C.

At 15°C, high O_2 concentrations decreased the rate of germination. GA applications partially relieved the inhibitory effect that high O_2 concentrations had on germination rate. Respiration rates were similar in 21 and 100% O_2, thus metabolic rates were not increased by higher O_2 concentrations. The negative effect of high O_2 may have been related to altered metabolic processes at the low temperature which did not kill the seeds since total germination was unaffected by increasing O_2 concentrations at 15°. High O_2 levels have been shown to reduce enzyme activity in bean embryos (12), inhibit cell elongation in wheat radicles (5), and promote proteolytic breakdown and enzyme activity in oats (1).

At 25°C, cyanide-sensitive and -resistant respiration were higher in 100 than in 21% O_2. However, at the end of the 6-day germination period, total respiratory activity was similar to that in air. At 15°, respiratory activity was similar, regardless of the concentration of O_2 in the environment. Cyanide-resistant respiration made up a small percentage of the total respiratory activity at both 15° and 25° regardless of O_2 treatment. Thus, alternate respiratory pathways were not related to the slowness of germination at 15° compared to 25°.

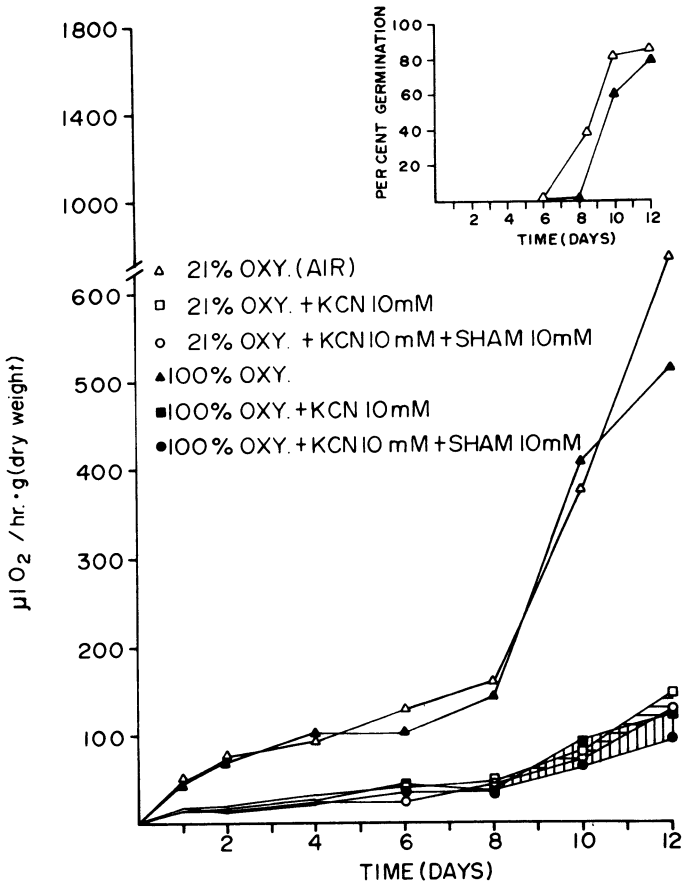

Fig. 5. Respiration and percent germination of pepper seeds germinated at 15° and 25°C with or without 100 ppm GA_{4+7}.

Yentur and Leopold (15) found that respiration in soybean seeds was dominated by cyanide-resistant respiration during the first 4 to 5 hr of germination. Gradually, after this time, the cytochrome pathway was predominant; at 9 hr nearly all respiration was cyanide-sensitive. In chick pea, however, Burguillo and Nicolas (2) reported that respiration was dominated by the cytochrome pathway for the first 12 hr of germination, shifting to more cyanide-resistant respiration which reached maximal activity after 72 to 96 hr. They also found that high O_2 concentrations could initiate the appearance of the alternate respiratory pathway. None of the variables (GA, O_2, or temperature) in the present experiments increased the respiration of pepper seeds via the alternate pathway. Thus, marked respiratory shifts could not explain the action of GA or high oxygen levels. McCaig and Hill (7) found that 100% O_2 did not stimulate the appearance of the alternate pathway in mitochondria isolated from etiolated wheat colcoptiles. Germination in 100% O_2 seemed to have little effect on either the cytochrome or cyanide-resistant pathways.

The addition of GA to pepper seeds stimulated germination and respiration rates. However, respiration rates of GA-treated seeds did not increase over those of untreated seeds until radicle emergence had already taken place. Therefore, GA does not appear to stimulate germination rates via respiratory-controlled metabolic activities prior to radicle emergence. Its action must occur during early germination leading to radicle elongation.

Lowering temperatures much below the optimum for germination can lead to severe reductions in germination rate. Decreasing the germination temperature from 25° to 15°C led to an increase in the mean days to germination of 'Early Calwonder' pepper seeds from 3.7 to 7.2, respectively. This was observed as a decrease in O_2 taken up (respiration) from about 550 µl O_2/hr·g (dry weight) of seed at 25° to 200 µl O_2 at 15° (at 50% radicle emergence). At 25°, germination rate was increased slightly by O_2 tensions up to 100%; however, respiration rates were increased almost 2-fold. At 15°, additional O_2 had a depressive effect on germination rate but did not alter total germination percentages. The addition of GA_{4+7} increased germination rate at either O_2 level, but was without effect on respiration rate until after radicle emergence occurred.

Thus, in addition to reduced respiratory rates, suboptimal temperatures appear to have an influence on germination of 'Early Calwonder' pepper seed at a level that may be under GA control. The nature of this control will be presented eleswhere.

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