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NOTE

Medical Oxygen Concentrators for Releasing Seed Dormancy

David M. Brenner,* Jack Dekker, Jarad Niemi, and Lisa Pfiffner

ABSTRACT

A new method is demonstrated for using concentrated oxygen (O_2 gas) to release seed dormancy. Concentrated O_2 gas in air is known to release seed dormancy in some seeds, including some foxtail (*Setaria*) species. New medical equipment makes O_2 gas easier to work with than before, so laboratories working with dormant seeds can now use concentrated O_2 gas as a seed treatment on a production basis. Use of medical O_2 gas concentrators is simpler and safer than using O_2 gas supplied by pressurized gas cylinders. Suitable medical O_2 gas concentrators in new or used condition are readily available, operate on standard electrical current, and deliver O_2 gas with low-pressure tubes and fittings. Resealable plastic bags are inflated with concentrated O_2 gas and then sealed as seed treatment chambers. This use of concentrated O_2 gas is confirmed to significantly increase the germination of dormant seeds of giant foxtail (*Setaria faberi* Herrm) and plains bristlegrass (*S. macrostachya* Kunth).

D.M. Brenner, Dep. of Agronomy, Plant Introduction Station, G212 Agronomy Hall, Iowa State Univ., Ames, IA 50011-1170; J. Dekker, Ames, IA 50014 (retired); J. Niemi, Dep. of Statistics, Iowa State Univ., Ames, IA 50011; and L. Pfiffner, USDA-ARS, Plant Introduction Research Unit, and North Central Regional Plant Introduction Station, Ames, IA 50011. Received 19 Nov. 2014. Accepted 23 Feb. 2015. *Corresponding author (dbrenner@iastate.edu).

Seed dormancy is an impediment to germplasm work with crop wild relatives. The beneficial effect of concentrated O_2 gas on releasing seed dormancy has been demonstrated. Dekker and Hargrove (2002) released dormancy of giant foxtail seeds with concentrated O_2 gas, increasing the germination frequency from 37% in air (21% O_2 gas) to 86% with 75% O_2 gas concentration. Similar dormancy release or stimulated germination is reported in other taxa: amaranth (*Amaranthus caudatus* L.) (Gutterman et al., 1992), chicory (*Cichorium intybus* L.) (Bradford et al., 2007), orchardgrass (*Dactylis glomerata* L.) (Probert et al., 1985), red pincushion-protea [*Leucospermum cordifolium* (Salisb. ex Knight) Fourc.] (Van Staden and Brown, 1973), rice (*Oryza sativa* L.) (Roberts, 1962), and cocklebur (*Xanthium strumarium* L.) (Shull, 1914; Thornton, 1935). The effect was reviewed by Bewley and Black (1994) and Corbineau and Côme (1995). Germination coincides with a rise in oxidative respiration (Bewley and Black, 1994; Bradford et al., 2013; Zhao et al., 2013). Although the dormancy-releasing effect of concentrated O_2 gas is common, it is not universal; for example, it was not found in switchgrass (*Panicum virgatum* L.) (Duclos et al., 2013). The use of concentrated O_2 gas as a dormancy-releasing seed treatment is now practical

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because new medical concentrators are available and are able to increase O_2 gas from the ambient concentration to 90% (McCoy, 2013). Such concentrators are safer and more convenient than pressurized gas cylinders.

The goals of this paper are to (i) demonstrate the practical use of medical O_2 gas concentrators to apply concentrated O_2 gas as a dormancy-releasing seed treatment and (ii) replicate the results of Dekker and Hargrove (2002) using the proposed O_2-concentrating apparatus.

MATERIALS AND METHODS

A test of the effectiveness of the O_2 concentrator was conducted by comparing three treatments of *Setaria* seeds in a germinator: (i) concentrated O_2 gas in sealed chambers ("oxygen"), (ii) ambient air from an aquarium air pump in sealed chambers ("air"), and (iii) traditional germination boxes without sealed chambers ("traditional"). The air and traditional treatments had ambient-sourced air. Oxygen was provided for the O_2 gas treatment by an Invacare 5 (Invacare, Elyria, OH) stationary medical O_2 gas concentrator. The functioning of the O_2 gas concentrator was verified with an Invacare Invirc400 concentrator analyzer, equilibrating the output at 96.3% O_2 gas on the day the chambers were filled. For the air treatment, an aquarium air pump was used to fill the sealed chambers. Our chambers were 45 by 57 cm vacuum sealed polyethylene and nylon airtight sealable bags (70 μm thickness; Ziploc Space Bag, S.C. Johnson & Son, Inc., Racine, WI). These bags have one-way vacuum valves, which remained closed. The chambers were inflated with standard 4.76-mm interior diameter medical O_2 gas supply tubing from the concentrator and through the bag's mouth. After the chamber was inflated, the tube was withdrawn quickly and the chamber was sealed. All chambers maintained positive pressure, remaining turgid (inflated to approximately 15 cm thick) until opened at the end of the experiment. The three seed lots examined were (i) PI 509035 plains bristlegrass seeds harvested in 2011 and (ii) PI 669940 giant foxtail seeds harvested in 2006, both from the USDA's North Central Regional Plant Introduction Station in Ames, IA, and (iii) giant foxtail seeds wild-collected on 10 Nov. 2013 in Ames, IA, with the collector's number DB 2013006. The seeds were stored at 4°C and 25% relative humidity. Each of two replications had 100 seeds from each of three seed lots, which were placed on 80-mm blue blotter paper (Anchor Paper Co., St. Paul, MN) in translucent polypropylene living hinge boxes measuring 14 by 14 by 3 cm (Alpack Plastic Packaging, Centerville, MA). The blotter paper was moistened with 0.1% KNO_3 in distilled water. The seeds imbibed in the treatment environments without pretreatments. They were incubated in one germinator (Conviron CMP 5000, Winnipeg, Manitoba, Canada) configured with six shelves. Each treatment was placed on two shelves in random order. The daily conditions in the germinator alternated between 16-h light periods at 30°C and 8-h dark periods at 20°C, following Dekker and Hargrove (2002). The lids of the boxes in the sealed chambers were not sealed but in the traditional treatment (no sealed chambers), the box lids were sealed. Germination was evaluated 8 d after seeds were placed in the chambers and was determined by radicle emergence through the seed coat.

Table 1. Germination results for three seed lots using traditional germination boxes, ambient air in sealed chambers, and concentrated O_2 gas in sealed chambers. Each germination test was replicated twice. Germination was measured after 8 d at 20°C (night, 8 hr) and 30°C (day, 16 hr).

		Setaria seed lots		
Treatments	Replications	PI 509035 *S. macrostachya*	PI 669940 *S. faberi*	DB 2013006 *S. faberi*
		— Germination counts per 100 seeds —		
Traditional germination boxes	1	26	13	23
	2	9	9	23
Ambient air in sealed chambers	1	15	13	23
	2	15	14	33
Oxygen in sealed chambers	1	41	29	49
	2	54	40	47

A Bayesian β-binomial model was used to estimate the germination probability for each treatment–seed lot combination using independent Jeffreys priors (Gelman et al., 2014); the posterior distribution of germination probability for each treatment–seed lot combination is a β distribution with the parameters 0.5 plus the number of successful germinations and 0.5 plus the number of unsuccessful germinations. We report estimates of these probabilities as 95% credible intervals. To estimate whether any given treatment was more effective than another, 1000 posterior samples were drawn and the probability that one treatment was more effective than another was estimated using the proportion of posterior draws that were larger for one treatment than another (Gelman et al., 2014).

RESULTS

The number of germinated seeds, out of 100, is shown in Table 1 by treatment, seed lot, and replicate; the ungerminated seeds were firm at the end of the study. For PI 509035, germination probability was estimated to be between 41 and 54% in the O_2 gas treatment, between 11 and 20% in the air treatment, and between 18 and 30% in the traditional treatment. For these seeds, the O_2 gas treatment was better than air ($P > 0.99$) and better than the traditional treatment ($P > 0.99$) and the traditional treatment appeared to be more effective than air ($P = 0.99$). For PI 669940, germination probability was estimated to be between 28 and 41% in the O_2 gas treatment and between 9 and 19% in the air treatment, and between 7 and 16% in the traditional treatment. In this seed lot, the O_2 gas treatment was better than air ($P > 0.99$) and better than traditional ($P > 0.99$) and air may have been more effective than the traditional treatment ($P = 0.77$).

For DB 2013006, germination probability was estimated to be between 41 and 55% in the oxygen treatment, between 22 and 35% in the air treatment, and between 18 and 29% in the traditional treatment. For these seeds, the O_2 gas treatment was better than air ($P > 0.99$) and better

than traditional ($P > 0.99$) and air may have been more effective than the traditional treatment ($P = 0.89$).

DISCUSSION

We replicated the dormancy-releasing effect of concentrated O_2 gas observed by earlier authors. Laboratories such as gene banks that work with dormant seeds of crops' wild relatives can benefit from concentrated O_2 gas applications to release dormancy. In addition to its use in seed dormancy release, the same apparatus could have application in the study of seed aging (Ohlrogge and Kernan, 1982, Groot et al., 2012), and may provide easily manipulated biologically available O_2 gas to improve the performance of aged low-vigor seeds as an alternative to the hydrogen peroxide used by Liu et al. (2012). Indeed the increased germination we observed may be partly from increased vigor and not just dormancy release. The O_2 gas concentrator can be readily scaled up from the design described here by use of larger plastic bags or other sealed chambers.

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