A method for germinating perennial *Cicer* species

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The germplasm collection at the USDA ARS National Plant Germplasm System includes chickpea that consists of cultivated *Cicer arietinum* accessions containing genetic diversity immediately accessible for breeding, and wild *Cicer* species that may be of importance in the future as sources of genes for resistance to biotic and abiotic stresses. The wild *Cicer* species in the collection include 113 accessions of seven annual wild *Cicer* species and 59 accessions of 13 perennial *Cicer* species (available online at www.ars-grin.gov/npgs). At USDA-ARS, located in Pullman, WA USA, in earlier years, seed aeration technique was used to promote germination in perennial *Cicer* species. N Kameswara Rao has used *in vitro* germination on water agar to uniformly germinate annual wild *Cicer* species (personal communication 2000).

In the present study, an *in vitro* germination method was examined as an alternative method to provide uniform germination of the perennial species with the goal of establishing a nursery for regeneration and evaluation of inter- and intra-accession genetic variability. Twenty-eight accessions of nine perennial species were surface disinfested, scarified, and cultured under sterile conditions on water agar. The average germination of 25 accessions of eight species was 82% in 2001 with a range of 43 to 100%. Two accessions of *C. montbretii* failed to germinate *in vitro*. In 2002, an additional 13 accessions were successfully germinated with the same method (data not shown) and three accessions of *C. montbretii* failed to germinate on water agar.

Seeds to be germinated were surface disinfested with a 30 sec dip in 95% ethanol followed by 10 min in 0.6% NaClO (100% commercial bleach) with drops of the detergent Tween 80 (Sigma, St. Louis, MO USA). After surface disinfestations, the seeds were soaked in sterile water for 1–5 days, or until they soften enough to scarify using a sterile scalpel. Scarified seed were then placed either on agar plus Murashige & Skoog (1962) Salt Mixture (M5524, Sigma, St Louis, MO USA) or on agar (Difco Bacto agar, Fisher Scientific, USA) medium alone. The culture vessels (Magenta GA-7-3, Sigma, St Louis, MO USA) were then placed under cool-white fluorescent lights until radicle emergence. Plantlets were left on agar until the shoots were between 2–5 cm long.

An experiment was conducted to test the effects of Murashige and Skoog (1962) mineral nutrients plus agar vs. unamended agar on germination. The percentage germination was similar; however, MS nutrient amended agar reduced root growth (data not shown).

The vessels containing the plantlets were then partially opened to begin a 2 or 3 day acclimation at lower relative humidity. Plantlets were then pulled from the agar and their roots dipped in a fungicidal slurry (Captan, Gustafson, Plano, TX USA) before transplanting to 18 cm flats (Rootrainers, Hummert, Earth City, MO USA) filled with soil-less planting mix (Sunshine Mix Aggregate Plus Blend #4, SunGro, Bellevue, WA USA) with added coarse perlite (#2 sieve, Therm-o-rock, Chandler, AZ USA). The planted flats were moved to a humidity-controlled chamber constructed on a greenhouse bench. Plastic sheeting, 67% light-reducing shade fabric (PAK Woven Shade Fabric, Hummert, Earth City, MO USA) and a humidifier (Model 500, Hummert, St. Louis, MO USA) were used to maintain a cooler atmosphere with constant relative humidity. Initial humidity settings were between 75–80% with a steady decline to approximately 50% over the course of 4 to 5 days. The seedlings were able to tolerate the ambient humidity after five days and were moved to an open greenhouse bench covered with shade fabric. The greenhouse conditions were 21°C day/15.5°C night temperature, no humidity control, and 16 h day length.

The *Cicer* plants were retained there for a few weeks and the plants grew robust enough to withstand the outdoor conditions. Later, the plants were moved to an outdoor lathe house to harden the seedlings for at least two weeks before planting in the field. The seedlings were hand-planted on either side of a central irrigation drip line with emitters next to each plant.

This procedure provided uniform germination of most of the perennial *Cicer* accessions, except *C. montbretii* (Table 1). Aseptic germination of perennial chickpea on water agar is a fast and efficient method to provide a uniform set of transplants for field regenerations, and also to offer sufficient uniform seedlings for replicated screenings to detect resistance to biotic and/or abiotic stresses. Once established, grafting may also be useful in supplying plants for resistance testing experiments (Chen

Table 1. Results of aseptic germination of perennial *Cicer* species on water agar after seed surface disinfestation and scarification at USDA-ARS, Pullman, in 2001.

Accession Number	Genus	Species	Seed quantity	Germinated	% Germinated	Number Rotted[1]	% Rotted
PI 383626	*Cicer*	*anatolicum*	30	24	80.0%	4	13.3%
PI 561078	*Cicer*	*anatolicum*	30	14	46.7%	8	26.7%
PI 599087	*Cicer*	*anatolicum*	30	17	56.7%	3	10.0%
PI 557453	*Cicer*	*canariense*	30	13	43.3%	0	0.0%
PI 599079	*Cicer*	*macracanthum*	30	23	76.7%	2	6.7%
PI 599080	*Cicer*	*macracanthum*	30	28	93.3%	1	3.3%
PI 599081	*Cicer*	*macracanthum*	30	25	83.3%	2	6.7%
PI 532928	*Cicer*	*microphyllum*	30	27	90.0%	3	10.0%
PI 593718	*Cicer*	*microphyllum*	30	28	93.3%	0	0.0%
PI 593719	*Cicer*	*microphyllum*	30	27	90.0%	1	3.3%
PI 599061	*Cicer*	*microphyllum*	30	27	90.0%	1	3.3%
PI 599082	*Cicer*	*microphyllum*	30	29	96.7%	0	0.0%
PI 599083	*Cicer*	*microphyllum*	30	30	100.0%	0	0.0%
PI 599084	*Cicer*	*microphyllum*	30	27	90.0%	0	0.0%
PI 599088	*Cicer*	*microphyllum*	30	24	80.0%	2	6.7%
PI 599089	*Cicer*	*microphyllum*	30	29	96.7%	0	0.0%
PI 599093	*Cicer*	*microphyllum*	30	28	93.3%	0	0.0%
PI 599085	*Cicer*	*multijugum*	30	27	90.0%	0	0.0%
W6 11516	*Cicer*	*multijugum*	30	30	100.0%	0	0.0%
PI 561084	*Cicer*	*oxyodon*	30	27	90.0%	0	0.0%
PI 561103	*Cicer*	*oxyodon*	30	25	83.3%	1	3.3%
PI 599053	*Cicer*	*songaricum*	30	13	43.3%	0	0.0%
PI 504550	*Cicer*	*yamashitae*	30	28	93.3%	1	3.3%
PI 510657	*Cicer*	*yamashitae*	30	30	100.0%	0	0.0%
PI 510664	*Cicer*	*yamashitae*	30	21	70.0%	1	3.3%
PI 599090	*Cicer*	*montbretii*	30	0	0.0%		
PI 599091	*Cicer*	*montbretii*	30	0	0.0%		

1. Seed was scored as rotted if contaminated *in vitro* with a micro-organism, usually fungal or bacterial in appearance.

et al. 2004). As reported by Kaiser et al. (1997), cold treatment followed by aeration of *C. montbretti* seed in fresh water failed to promote germination. At ICRISAT, Patancheru, India, seedlings of C. *montbretii* were routinely established following normal germination procedures. (Anonymous communication), and plants of *C. montbretti* were established at Pullman, WA USA, in the early 1990s using an unpublished germination procedure (Hellier, personal communication). Further research is needed to improve surface disinfestation of the seed to reduce losses from fungal and bacterial contamination (rotted seed), and compare this method with (1) aeration in fresh water and (2) ICRISAT procedures in germination efficiency and efficacy. A technique to achieve seed germination in C. *montbretii* has to be developed.

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