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W. W. Roath
Iowa State University

M. P. Widrlechner
United States Department of Agriculture, isumw@iastate.edu

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Inducing germination of dormant *Cuphea* seed and the effects of various induction methods on seedling survival

W. W. ROATH and M. P. WIDRLECHNER

United States Department of Agriculture, Agricultural Research Service, Plant Introduction Station, Iowa State University, Ames, Iowa, 50011, USA

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Summary

Cuphea wrightii Gray, and *C. laminuligera* Koehne are potential domestic sources of medium-chain-length fatty acids. These species have many undesirable characters including post harvest dormancy in which seeds remain dormant for one year or more. Researchers have shown that common techniques to induce germination in these species are not effective. Seed lots from these species were produced in 1983 and 1986. Excised seed (seed coats removed) and nonexcised seed (seed coats intact) were germinated on unsupplemented agar medium and on paper germination towels. No germination was obtained from nonexcised 1986 *C. wrightii* seed. Germination of 1% or less was obtained from nonexcised 1986 *C. laminuligera* seed. Excising seed coats from 1986 seed improved germination to over 81% and improved 1983 seed germination from 40% to 92%. Germination of seed (averaged over treatments) on agar medium (54%) was significantly greater than on towels (44%). Seedling survival after 21 days in pots on a greenhouse mist bench was higher for excised seed (up to 68%) than for unexcised seed (up to 53%) for 1983 seed. Germinating excised seed on agar medium can be a useful technique to germinate dormant *Cuphea* seed.

Résumé

Induction de la germination des semences dormantes de Cuphea *et influence de différentes méthodes de stimulation sur la survie des plantules*

Cuphea wrightii Gray et *C. laminuligera* Koehne sont des sources potentielles d'acides gras à chaînes de longueur moyenne. Ces espèces ont de nombreux caractères gênants dont une dormance des semences qui se maintient pendant un an ou plus. Les chercheurs ont montré que les techniques habituelles d'induction de la germination ne sont pas efficaces. Les lots de semences de ces espèces ont été produits en 1983 et 1986. Les semences excisées (après ablation des téguments) et non excisées (avec les téguments intacts) ont été mises à germer sur de l'agar non enrichi et sur du papier. Aucune germination n'a été obtenue avec les semences intactes de *C. wrightii* récoltées en 1986. La germination ne dépassait pas 1% avec les semences intactes de *C. laminuligera* récoltées en 1986. L'ablation des téguments permettait d'atteindre jusqu'à 81% de germination avec les semences de 1986 et 40 à 92% avec celles de 1983. La germination des semences (en moyenne après les traitements) sur agar (54%) était significativement plus élevée que sur papier (44%). La survie des plantules, après 21 jours dans des pots placés en serre sous brouillard, était meilleure avec les semences excisées (jusqu'à 68%) qu'avec les semences intactes (jusqu'à 53%) de 1983. Les essais sur agar avec les semences excisées peuvent constituer une technique utile pour la germination des semences dormantes de *Cuphea*.

Zusammenfassung

Die Keiminduktion bei dormanten Cuphea-*Samen und der Einfluß verschiedener Induktionsmethoden auf das Überleben der Keimlinge*

Cuphea wrightii Gray und *C. laminuligera* Koehne sind mögliche einheimische Quellen für Fettsäuren mittlerer Kettenlänge. Diese Arten besitzen viele unerwünschte Eigenschaften, darunter eine Nacherntedormanz, bei welcher die Samen ein Jahr lang oder gar länger dormant bleiben. Forscher wiesen nach, daß die gängigen Verfahren einer Keiminduktion bei diesen Arten nicht wirksam sind. Saatgutpartien dieser Arten wurden 1983 und 1986 produziert. Herauspräparierte Samen (Samenschalen entfernt) und nicht präparierte Samen (intakte Samenschalen) wurden auf reinem Agar-Substrat und in Papierrollen eingekeimt. Bei nicht herauspräparierten Samen von *C. wrightii* von 1986 wurde keine Keimung erhalten. Eine Keimfähigkeit von 1% oder weniger zeigten nicht präparierte Samen von *C. laminuligera* von 1986. Die Entfernung der Samenschalen der Samen von 1986 verbesserte die Keimfähigkeit auf über 81% und verbesserte die Keimfähigkeit der Samen von 1983 von 40% auf 92%. Die Keimfähigkeit der Samen (gemittelt über die Behandlungen) war auf dem Agar-Substrat mit 54% signifikant höher als in den Rollen mit 44%. Bei den Samen von 1983 war die überlebenshäufigkeit nach 21 Tagen in Töpfen auf einem Sprühtisch im Gewächshaus bei den herauspräparierten Samen mit bis zu 68% höher als bei den nicht herauspräparierten Samen mit bis zu 53%. Das Keimen herauspräparierter Samen auf Agar-Substrat kann als brauchbares Verfahren zur Einkeimung dormanter Samen von *Cuphea* angesehen werden.

Introduction

Cuphea wrightii Gray and *C. laminuligera* Koehne are promising domestic sources of lauric acid, a medium-chain-length triglyceride used in the manufacture of soaps and detergents (Graham, Hirsinger and Robbelen, 1981; Thompson, 1984; Wolff, Graham and Kleiman, 1983). These species are undomesticated and have many characteristics that impose serious agronomic constraints. One of these traits is prolonged seed dormancy. Treatments such as heating, chilling, mechanical scarification, and growth-regulators had little, or in some cases, negative effects on germination of less than one-year-old seed (Grabe, Garbocik and Kaliangile, 1985). Soaking in concentrated sulphuric acid for 10 minutes gave up to 63% germination of less than one-year-old *C. wrightii* seed (Grabe *et al.*, 1985). After freshly harvested *C. wrightii* embryos were placed on agar medium supplemented with Murashige and Skoog salts, 31% germination was obtained, and 63% was obtained when the medium was supplemented with 1.0 or 10.0 mg/L BA (6-benzyl-aminopurine) (Janick and Whipkey, 1986). Removing seed coats of sulphuric acid treated ungerminated seed further improved the germination percentage (Thompson, 1984).

This study was conducted to evaluate germination media and the effects of excising seed coats on seed germination and seedling survival of less than one-year-old and of three-year-old *C. wrightii* and *C. laminuligera* seed.

Materials and methods

C. wrightii and *C. laminuligera* seed were produced at Corvallis, Oregon in 1983 and at Ames, Iowa in 1986. Media supplemented with Murashige and Skoog salts (Janick

and Whipkey, 1986) and consisting of 8.0 g/L agar (Difco Bacto-agar, Detroit, Michigan, USA[1]) in distilled water only were autoclaved and poured into sterile disposable petri dishes. Seed was soaked in sterile distilled water overnight. Twenty-five seeds each of excised (seed coats removed) and unexcised seed were germinated on agar medium and on Anchor Paper[1] germination towels. Each of these treatments was replicated eight times. Seeds were placed in a germination cabinet with alternating 12 hour periods of 21 °C and 31 °C temperatures and 16 hours of light. Percentage germination was determined at the end of seven days. Seedlings were transplanted into pots containing commercial potting soil and then the pots were placed on a mist bench in the greenhouse. The greenhouse temperature was 21 °C and supplemental light from metal-halide high intensity lamps was provided for 14 hours per day. Percentage seedling survival was determined at the end of 21 days as the number of surviving seedlings per number of seeds germinated.

A randomised complete block, four-factor analysis of variance was used to determine differences between treatment means.

Results and discussion

Germination of seeds on agar medium was significantly better than germination on paper towels (table 1). There was virtually no germination of 1986 nonexcised seed (table 2). Removing seed coats from 1983 and 1986 seed improved germination. Germination of 1986 excised seed on agar was significantly better than on paper towels (table 2). The significant reduction in germination of *C. wrightii* 1983 nonexcised seed on agar was probably due to fungal contamination of the agar. Over all treatments, there was no difference in percentage germination between the two species (table 1).

No significant difference was detected in germination of *C. wrightii* seed on agar medium supplemented with Murashige and Skoog salts versus unsupplemented agar medium. We found it more difficult to control fungal and bacterial growth on the mineral supplemented medium. These factors caused us to discontinue its use.

It is apparent that there is a growth inhibitor associated with the seed coat in these two species which is affecting germination of seed that is at least three years old. The nature of this inhibition is not known at this time, but it is apparently unaffected by most common treatments used to break dormancy (Grabe *et al.*, 1985). Treatment with sulphuric acid will, apparently, partially deactivate the inhibitor.

The percentage seedling survival though considerably lower than germination, was highly correlated to percentage germination ($r = 0.82$, statistically significant at $p = 0.05$). Over all treatments, *C. wrightii* had a significantly higher percentage seedling survival than *C. laminuligera* (table 3). The benefit of seed coat removal on germination of 1983 and 1986 *C. wrightii* seed is apparent in seedling survival (tables 2 and 4). This was not true for *C. laminuligera* 1983 seed. Seedlings from 1983 excised seed

[1] The mention of commercial products is not to be construed as an endorsement of any product by USDA-ARS or its cooperators.

from this species appeared weaker than those seedlings from unexcised seed and fewer survived.

These data indicate that germinating new, excised seed of these two *Cuphea* species on agar media is necessary to assure an adequate number of seedlings. The relatively high percentage mortality will have to be considered in determining the number of seeds to germinate. Because of the tediousness of excising seed coats and the high mortality, it is doubtful whether it would be helpful to excise seed coats from older seed. The amount of seed available, the species under investigation, and the intended use of the plants would have to be considered before deciding to apply this procedure to older seed.

The procedure of excising seed coats from new seed of these two *Cuphea* species and germinating the excised seed on agar media will allow researchers to obtain plants from dormant seed.

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Table 1. Mean germination of two *Cuphea* species and of seed germinated on selected media.

Species	Percentage germination
C. wrightii	49a
C. laminuligera	49a
Medium	**Percentage germination**
Agar	54a*
Towels	44b

*Entries followed by different letters are significantly different (lsd. p = 0.05).

Table 2. Mean germination of 1983 and 1986 seed of two *Cuphea* species on selected media.

Species	Seed	Percentage germination			
		1983 seed		1986 seed	
		Agar	Towel	Agar	Towel
C. wrightii	excised	98a*	91ab	78d	34g
C. wrightii	nonexcised	29g	66e	0h	0h
C. laminuligera	excised	87bc	80cd	86bcd	30g
C. laminuligera	nonexcised	52f	55f	1h	1h

*Entries followed by different letters are significantly different (lsd. p = 0.05).

Table 3. Mean seedling survival of two *Cuphea* species and of seed germinated on selected media after 21 days on a greenhouse mist bench.

Species	Percentage seedling survival
C. wrightii	32a*
C. laminuligera	22b
Medium	**Percentage seedling survival**
Agar	31a*
Towels	24b

*Entries followed by different letters are significantly different (lsd. p = 0.05).

Table 4. Mean seedling survival of two *Cuphea* species after germination of 1983 and 1986 produced seed on selected media.

Species	Seed	Percentage survival			
		1983 seed		1986 seed	
		Agar	Towel	Agar	Towel
C. wrightii	excised	65a*	68a	42cd	15g
C. wrightii	nonexcised	18fg	53bc	0h	0h
C. laminuligera	excised	29ef	18gd	62ab	14g
C. laminuligera	nonexcised	33de	23efg	1h	1h

*Entries followed by different letters are significantly different (lsd. p = 0.05).

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