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Scratching the surface: The *in vitro* research that will be critical for conserving exceptional plants to scale

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Abstract
The conservation of threatened exceptional plants, which cannot be conserved by seed banking, requires *in vitro* technologies for many of the approaches needed for their long-term *ex situ* conservation. This study evaluated the current *in vitro* plant literature, as represented in Web of Science, to determine its taxonomic overlap with the families and genera of the 775 species currently listed as exceptional. Web of Science was searched using the terms micropropagation, somatic embryogenesis, zygotic embryo, and cryopreservation, and the target genera and families were identified in the more than 19,000 articles evaluated. There were five families with significant overlap between the *in vitro* literature and exceptional species: Fabaceae, Asteraceae, Orchidaceae, Arecaceae, and Rutaceae. However, there was less overlap at the level of genus, with *Citrus, Coffea,* and *Quercus* having the most articles. Significant gaps were also found, with 14 exceptional families and half of the exceptional genera having no representation in the Web of Science search results. The 20 exceptional species with the most articles were all economically important species, and these had 343 threatened congeners that could be prioritized for research. A highly important group of exceptional plants that was significantly under-represented in the literature was tropical woody species, which form the backbone of the diversity of the world's threatened rainforests. Overall, there are areas of strength upon which to build future work, but significant gaps where research should be prioritized for effectively conserving exceptional plants.

Keywords Conservation · Exceptional plant · *In vitro* · Literature · Meta-analysis

Introduction

Over the past several decades, *in vitro* methods for plants have been critical for the development of current biotechnologies used for plant improvement, as well as for the horticultural and plant products industries (Rout *et al*. 2006; Altman 2019; Gubser *et al*. 2021), and hundreds of species have been initiated into culture. It is now also becoming evident that *in vitro* plant methods will be necessary for conserving many threatened plant species that are not adaptable to seed banking, for example exceptional species (Pence 2014).

The scope of this challenge is significant. While the total number of exceptional species is not known, there are estimates for certain types of exceptional species, particularly those that have desiccation sensitive seeds (recalcitrant species). It is estimated that 8% of the global flora will fall into this category and that a high proportion of these species will be woody species from the tropics (Tweddle *et al*. 2003; Wyse and Dickie 2017). Based on a total flora of about 383,000 species (World Flora Online 2023), this would represent over 30,000 species. It is estimated that 45% of all flora are threatened (Antonelli *et al*. 2023), and thus, this suggests that over 13,000 species would be classified as threatened and exceptional, requiring methods beyond conventional seed banking for effective, long-term conservation. Estimates of other types of exceptional species are more difficult to make, although analyses of short-lived seeds have suggested that these will likely also number into the thousands (Colville and Pritchard 2019).

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Cryobiotechnologies have been identified as one of the primary tools that will be needed to meet the challenge of the long-term *ex situ* conservation of exceptional plants (Pence *et al*. 2020). The methods required to accomplish this will vary depending on the cause of the species' exceptional status, identified by its exceptionality factor (EF), but many of the approaches will require *in vitro* technologies (Table 1.). For species with few or no seeds available (EF1), dormant buds or *in vitro* tissues will be needed for cryobanking. There are several methods for recovering dormant buds, depending on the species, but in some cases *in vitro* growth or *in vitro* grafting is required (Matsumoto *et al*. 2015; Tanner *et al*. 2021). For *in vitro* tissues (shoot tips or somatic embryos) to be used for cryopreservation, methods must be in place to initiate and grow these tissues and then to recover and grow them into plants after freezing (Krajňáková *et al*. 2013; Zhang *et al*. 2023). For exceptional species with available seeds, but which cannot survive the drying or long-term freezing of seed banking (some EF2 and EF3), isolated zygotic embryos may be adaptable to cryopreservation (Ballesteros *et al*. 2021). When this is possible, *in vitro* procedures are needed to recover and germinate the embryos post-thawing (Gaidamashvili *et al*. 2021). Deeply dormant seeds (EF4) often do not require *in vitro* methods, although in some cases isolating embryos from the seeds has been helpful in achieving germination (Dolce *et al*. 2015). In all of these cases, there must be *in vitro* protocols in place that are consistently successful to effectively conserve the species that will rely on them.

In a recent study, the plant cryopreservation literature was examined to assess the status of the field and identify gaps in the taxonomic breadth of the application of cryopreservation technologies to exceptional plants (Pence and Bruns 2022). The study reported here was undertaken to similarly examine the alignment of current knowledge on *in vitro* plant culture with the needs of exceptional species conservation. A search of the Web of Science literature was made for *in vitro* plant research, and this was compared with the current Global Working List of Exceptional Plants (Pence *et al*. 2022), hereafter referred to as the "List of Exceptional Plants", and available online at http://cincinnatizoo.org/epcn. The goal was to highlight the importance of *in vitro* methods for conserving exceptional plants and to identify gaps that may need to be addressed to achieve effective long-term conservation of the thousands of species predicted to be exceptional.

Table 1. An overview of the methods needed for exceptional plant conservation, indicating where *in vitro* methods (shaded cells) will be needed to support cryopreservation; organized by exceptionality factor (EF)

Exceptionality Factor[a]	Storage tissue	Storage method	Recovery method(s)
EF1 (seeds unavailable)	*In vitro* tissues (shoot tips, somatic embryos)	Cryopreservation	*In vitro*
	Dormant buds	Cryopreservation	*In vitro* culture, grafting
EF2 (desiccation sensitive)	Embryo	Cryopreservation	*In vitro*
	In vitro tissues (shoot tips, somatic embryos)	Cryopreservation	*In vitro*
	Dormant buds	Cryopreservation	*In vitro* culture, grafting
EF3 (freeze-sensitive, short-lived)	Seeds	Cryopreservation	Germination
	Embryo	Cryopreservation	*In vitro*
EF4 (deeply dormant)	Seeds	Seed banking (-20°C)	*In vitro,* other

[a]Reasons for exceptionality, as described in Pence *et al*. 2022

Materials and methods

The process of searching the literature and preparing the resulting files for analysis was based on methods used for a similar analysis of the cryopreservation literature (Pence and Bruns 2022). More details on the process can be found there.

Biosis Previews on Web of Science (Clarivate Analytics, London, U.K.) was accessed through the University of Cincinnati Libraries on 23 February 2023 and searches were made for "micropropagation" (10,519 results), "somatic embryogenesis AND plant" (10,256 results), and "zygotic embryo AND vitro AND plant" (1,037 results). Additionally, search results were also included for "cryopreservation AND plant" (2,424 results) and "cryopreservation AND seed" (1,277 results), which were downloaded on 11 March 2022. These records were analyzed in Pence and Bruns (2022) from a cryopreservation perspective, but they were analyzed here based on any *in vitro* methods they described.

The Web of Science searches were downloaded as Excel files, which were imported into R for analysis (R Core Team 2023); the script used for all data analysis is available at https://github.com/eb-bruns/WoS_wordsearch_invitro. Using the Web of Science IDs, duplicate articles were removed. This resulted in 22,251 articles. To identify genus and family names in the article titles and abstracts, the World Flora Online (WFO) Taxonomic Backbone (v.2021.12) (World Flora Online 2021) was used to create lists of genera and families, and functions from the stringr R package (Wickham 2022) were used to find matches. This analysis resulted in 1,739 articles with no genus identified and 20,083 articles with no family identified. But, since a family or families could be assigned for articles with a genus

Table 2. The top 25 families with the most Web of Science articles identified, compared with the 25 families on the Global Working List of Exceptional Plants (Pence *et al.* 2022) with the largest number of species currently known to be exceptional. Shaded families are those in common in the top 25 of each list; *starred* (*) families are those in common in the top 15 of each list

Literature Search		Exceptional Plant List		
Family	Number of Articles	Family	Number of Species	Number of Articles
Poaceae	1651	Dipterocarpaceae	59	4
Rosaceae	1331	Arecaceae*	37	460
Fabaceae*	1301	Rutaceae*	35	369
Pinaceae	907	Campanulaceae	34	28
Asteraceae*	878	Fabaceae*	32	1301
Solanaceae	788	Rubiaceae	32	264
Orchidaceae*	693	Orchidaceae*	26	693
Brassicaceae	618	Meliaceae	25	108
Apiaceae	469	Fagaceae	24	275
Arecaceae*	460	Lauraceae	24	75
Lamiaceae	453	Sapindaceae	23	130
Vitaceae	449	Asteraceae*	22	878
Musaceae	401	Primulaceae	20	94
Rutaceae*	369	Amaryllidaceae	18	316
Malvaceae	348	Moraceae	18	120
Myrtaceae	346	Myrtaceae	18	346
Asparagaceae	317	Rhizophoraceae	15	1
Amaryllidaceae	316	Apocynaceae	14	274
Bromeliaceae	307	Malvaceae	12	348
Euphorbiaceae	287	Sapotaceae	12	20
Fagaceae	275	Poaceae	11	1651
Apocynaceae	274	Anacardiaceae	10	146
Ericaceae	269	Araucariaceae	10	51
Rubiaceae	264	Gesneriaceae	10	63
Cucurbitaceae	255	Pittosporaceae	10	2

Table 3. Families in the Global Working List of Exceptional Plants, but with no articles identified in the Web of Science literature search

Family	Woodiness and Distribution	Number of Exceptional Species	Exceptionality Factor[a]			
			EF1	EF2	EF3	EF4
Asteliaceae	Non-woody; Australia, New Zealand, Pacific Islands, Mauritius, Reunion, S. South America (Birch *et al*. 2012)	1			x	
Atherospermataceae	Woody; Australia, New Caledonia, New Zealand, Chile (Renner *et al*. 2000)	1		x		
Chrysobalanaceae	Woody; New and Old World tropics (Prance and White 1988)	2		x		
Dipentodontaceae	Woody; tropical Asia, Australia, Pacific, extending to temperate E. Asia, tropical America (Ma and Bartholomew 2023)	1			x	
Griseliniaceae	Woody; New Zealand, South America (Dillon 2018)	1		x		
Gunneraceae	Non-woody; Southern hemisphere, subtropical, wet temperate (Fuller and Hickey 2005)	1			x	
Joinvilleaceae	Non-woody; Southeast Asia, Pacific Islands (Wysocki *et al*. 2016)	1			x	
Olacaceae	Woody; tropical (Malécot *et al*. 2004)	1		x		
Putranjivaceae	Woody; tropical (Kadiri and Muellner-Riehl 2021)	1		x		
Rafflesiaceae	Non-woody; tropical Southeast Asia (Bendiksby *et al*. 2010)	1	x			
Ripogonaceae	Woody; Australia, New Zealand, New Guinea (Conran *et al*. 2018)	1		x		
Surianaceae	Woody; Mexico, Southern Hemisphere (Schneider 2007)	1		x		
Tetrameristaceae	Woody; Central and South America, Southeast Asia (Kubitzki 2004)	1		x		
Zosteraceae	Non-woody; Marine, temperate, Northern and Southern Hemispheres (Kuo and Hartog 2001)	2		x		
Total		**16**	**1**	**9**	**4**	**0**
Percent			**6%**	**56%**	**25%**	**0%**

[a]Reasons for exceptionality, as described in Pence *et al*. 2022; EF1 = few or no seeds; EF2 = desiccation sensitive seeds; EF3 = short-lived seeds; EF4 = seeds deeply dormant

or genera identified, a total of only 1,629 articles ultimately had no family identified. Article titles and abstracts were also searched using a list of exceptional species names based on the List of Exceptional Plants; both the accepted names and synonyms on the list were used. The genus, family, and exceptional species names found were spot-checked and edited manually. To further confirm the articles were focused on *in vitro* culture of plants, article titles and abstracts were also searched for lists of manually curated keywords, as described in Pence and Bruns (2022).

Finally, publisher and author countries were analyzed. Using R and the countrycode R package (Arel-Bundock *et al*. 2018), the Web of Science publisher address and author addresses columns were analyzed to determine the country or countries. For authors, a list of unique countries was determined for each article, meaning results represent countries with at least one author and not necessarily a one-to-one list of each author's country. Results were manually reviewed to assign countries when no country was automatically identified.

To investigate the coverage of Web of Science, similar searches were made for a few families and genera that had poor representation in the Web of Science searches using Google Scholar (https://scholar.google.com/) on 25 July 2023. Searches were made for each family or genus AND "micropropagation," the family or genus AND "somatic embryogenesis," and the family or genus AND "zygotic embryo," and the first four pages of the results of each search were examined manually for relevant articles.

Results

The Web of Science searches resulted in 22,251 published articles. Several keywords were used to remove reviews and non-plant articles, leaving 19,117 articles dealing with *in vitro* work with plants, with 18,935 of those dealing with seed plants. These reported some aspect of *in vitro* culture for 257 families of seed-bearing plants. This list of articles was compared with the List of Exceptional Plants, which currently includes 111 families (Pence *et al*. 2022). For convenience, these are hereafter referred to as "exceptional families," although some species within the family may not be exceptional. The five families with the most articles on *in vitro* work were Poaceae, Rosaceae, Fabaceae, Pinaceae, and Asteraceae, while the five families with the most exceptional species were Dipterocarpaceae, Arecaceae, Rutaceae, Campanulaceae, and Fabaceae (Table 2). The two datasets

Table 4. The top 25 genera with the most Web of Science articles identified, compared with the 23 genera with six or more species listed in the current Global Working List of Exceptional Plants (Pence *et al*. 2022). Shaded genera are those in common between the two lists; *starred* (*) genera are those in common in the top 15 of each list

Literature Search			Exceptional Plant List			
Genus	**Number of Articles**	**Approximate Percent Trees[b]**	**Genus[a]**	**Number of Species**	**Number of Articles**	**Approximate Percent Trees[b]**
Solanum	461	16%	*Shorea*	26	2	89%
Vitis	443	0%	*Cyanea*	20	0	36%
Musa	393	0%	*Quercus*	17	173	59%
Prunus	378	19%	*Artocarpus*	15	17	98%
Pinus	376	60%	*Melicope*	15	0	51%
Picea	354	59%	*Coprosma*	12	0	38%
Arabidopsis	335	0%	*Lysimachia*	12	6	0%
Daucus	312	0%	*Dipterocarpus*	11	1	99%
*Citrus**	290	29%	*Citrus**	10	290	29%
Triticum	263	0%	*Cyrtandra*	10	0	6%
Malus	249	16%	*Pittosporum*	10	2	52%
Oryza	231	0%	*Araucaria*	9	49	67%
Medicago	228	1%	*Hopea*	9	1	94%
Brassica	210	0%	*Inga*	9	3	84%
Saccharum	208	0%	*Syzygium*	9	20	91%
Rosa	200	0%	*Clermontia*	8	0	76%
Eucalyptus	191	85%	*Aesculus*	7	26	50%
Gossypium	188	14%	*Garcinia*	7	23	78%
Coffea	187	75%	*Bruguiera*	6	1	56%
Zea	187	0%	*Coffea*	6	187	75%
Fragaria	179	0%	*Diospyros*	6	23	98%
Quercus	173	59%	*Rhizophora*	6	0	40%
Allium	167	0%	*Trichilia*	6	10	74%
Cucumis	166	0%				
Phoenix	166	40%				
	Average	**19%**			**Average**	**63%**

[a]There are eight genera with five species on the exceptional plant list, therefore these are not shown in the table

[b]Approximate percent trees is calculated for each genus based on the number of species listed in the BGCI GlobalTreeSearch database (BGCI 2023) divided by the number of species in World Flora Online designated as "Accepted" or "Unchecked" (v.2021.01)

had 12 families in common among the top 25 families and five in common in the top 15: Fabaceae, Asteraceae, Orchidaceae, Arecaceae, and Rutaceae. However, there were 14 families, each with one or two currently known exceptional species, that were not represented in the literature search at all (Table 3.). Most of these species are tropical or woody, with many from the Southern Hemisphere or marine. Over half of those species identified as exceptional in these families are exceptional because of seed desiccation sensitivity.

This study also yielded a list of 1,850 genera found within the *in vitro* literature and compared them to the 366 genera on the List of Exceptional Plants. These are hereafter referred to as “exceptional genera,” although not all species in a genus may be exceptional. The five genera with the most literature were *Solanum, Vitis, Musa, Prunus,* and *Pinus* while the five genera with the most exceptional species were *Shorea, Cyanea, Quercus, Artocarpus,* and *Melicope*. When the 25 genera with the most literature were

Table 5. Genera with six or more species listed in the Global Working List of Exceptional Plants (Pence *et al.* 2022), but no Web of Science articles identified

Genus	Woodiness		Climate			Native Distribution					Citation	Number of Exceptional Species	Exceptionality Factor[a]			
	Non-woody	Woody	Temperate	Subtropical	Tropical	Global	SE Asia	Pacific Islands	New Zealand	Hawaii			EF1	EF2	EF3	EF4
Cyanea		x			x					x	Oppenheimer 2020	15			15	
Melicope		x			x		x	x			Appelhans *et al.* 2018	16	14		1	1
Coprosma		x			x			x	x		Cantley *et al.* 2016	12			12	
Cyrtandra		x			x		x	x			Olivar *et al.* 2022	10	1		9	
Clermontia		x			x					x	Givnish *et al.* 2013	8			8	
Rhizophora		x			x	x					Takayama *et al.* 2021	6		6		
Total												**67**	**15**	**6**	**45**	**1**
Percent													**22%**	**9%**	**67%**	**1%**

[a]Reasons for exceptionality, as described in Pence *et al.* 2022; EF1 = few or no seeds; EF2 = desiccation sensitive seeds; EF3 = short-lived seeds; EF4 = seeds deeply dormant

compared with the 25 genera with the most exceptional species, only the genera *Citrus, Coffea,* and *Quercus* were common to both. Of the total 366 exceptional genera, 186 (51%) were not represented in the literature, including six genera that were among the 25 with the most exceptional species: *Cyanea, Melicope, Coprosma, Cyrtandra, Clermontia,* and *Rhizophora* (Tables 4. and 5.). The percentage of trees for each of the 25 top genera was estimated using the BGCI GlobalTreeSearch database (BGCI 2023), and the top 25 exceptional genera had an average of 63% of their species as trees while the top 25 genera from the literature averaged 19% (Table 4.). The six exceptional genera with no representation in the literature were all woody, tropical genera, dominated by species with short-lived seeds or having few or no seeds available for banking (Table 5.).

For the five families in common in the top 15 families of each list, the number of genera in common between the two lists within each of those families ranged from 20% (Asteraceae) to 68% (Orchidaceae) (Table 6.). The Fabaceae, with 1,301 articles, were represented by a large number of genera in the literature search (125) and also had a large number of exceptional species (24). Half (12) of these were represented in the literature. Orchidaceae, another exceptional family with a large amount of literature (693 articles) had information on 99 genera or genus hybrids. Of the 19 exceptional genera in the Orchidaceae, 13 (68%) were represented in the literature. In contrast, Asteraceae had 128 genera represented in the literature, but only three were in common with the 15 exceptional Asteraceae genera.

There were 20 species from the List of Exceptional Plants represented by 25 or more articles in the literature (Table 7.). Most of these are woody, tropical species with 10 having desiccation sensitive seeds (EF2) and 10 having short-lived seeds (EF3). They are all economically important, and in some cases represent the primary focus of all the articles in their family. Four species, *Carica papaya, Passiflora edula, Araucaria angustifolia,* and *Persea americana* were the subject of more than half of all the articles in their respective families. While six of these species had no threatened congeners, there were 343 threatened congeners for the remaining 14 species.

The Web of Science download also contained information on publisher and author addresses, and these were used to create heat maps, illustrating the distribution of the publishers and authors globally (Figs. 1 and 2). The largest number of articles came from publishers in the United States (24%), the Netherlands (21%), Belgium (9%), the U.K. (8%), and Germany (6%). A list of unique author countries was determined for each article, with the largest number of articles having at least one author from India (14%), the United States (10%), China (9%), and/or Brazil (7%). There were also 7% of articles for which no affiliation was given.

Although Biosis Previews on Web of Science includes over 5,300 journals in its database (https://mjl.clarivate.com/

Table 6. Genera in common between the Global Working List of Exceptional Plants (Pence *et al*. 2022) and the Web of Science literature search, for the five families in common in the top 15 families on the List of Exceptional Plants and the top 15 families with the most articles from the literature search

Family	Exceptional Species Genera	Literature Search Genera	Number in Common	Percent Exceptional Species Genera in Literature
Fabaceae	24	125	12	50%
Arecaceae	23	25	10	43%
Orchidaceae	19	99	13	68%
Asteraceae	15	128	3	20%
Rutaceae	6	32	4	67%

search-results), it is not a complete source of global literature. Google Scholar (https://scholar.google.com/) was used to further investigate the literature for Chrysobalanaceae and Zosteraceae, families with two species each on the List of Exceptional Plants but with no literature in the Web of Science search, as well as for Dipterocarpaceae, the family with the most species on the List of Exceptional Plants but with only four articles found in Web of Science. There were no articles for Zosteraceae and only one article for Chrysobalanaceae found this way, but 26 articles were found for Dipterocarpaceae that had not been found in the Web of Science search (Table 8.). These were conference and workshop proceedings, reports, a thesis, and journals. Some of these journals are not included in the Web of Science database, although five of them are, and the articles from those journals, while missed in the original searches, were found in the Web of Science database by searching for their titles. Several did not have titles or keywords that matched the original search words, and thus had not been found. A similar search of Google Scholar was made for the six genera in the top 25 exceptional genera that were not represented by any articles in the Web of Science search (Table 9.), and 14 articles were found with at least one for each genus except *Coprosma*. The types of sources were similar to those found for the Dipterocarpaceae, including a few articles from journals included in the Web of Science that were missed in the original search.

Although seed plants were the focus of this project, the literature search also retrieved articles on non-seed plants. The largest number of articles captured were on *in vitro* culture of algae (78), followed by pteridophytes (67) and then bryophytes (37) (Fig. 3). These represented 20, 14, and 17 families, respectively, and averaged less than two genera per family, indicating that research has been spread over a wide range of taxa within these groups (Table 10.).

Discussion

This study investigated the literature on *in vitro* plant culture to determine how well it currently supports the conservation of exceptional species. The literature was examined using the Web of Science with four key word searches to retrieve over 19,000 articles. Publishers for these articles were primarily from the United States and northern Europe, but one quarter of the articles had authors from India, China, and Brazil, countries with high levels of plant diversity. Previous work showed that many of the known exceptional species are native to South and Southeast Asia, including India, although also including a number of other countries that had less representation in this literature search (Pence *et al*. 2022). This current study agrees with the findings of Marks *et al*. (2023), who analyzed literature on plant science more broadly and found a similar trend in publishers, as well as the focus on economically important species that was evident in the current study's analysis.

Taxa reported in the articles from the Web of Science were aligned with the 775 species on the List of Exceptional Plants. The comparison of these two lists revealed that in some areas there is a good foundation for future work, particularly with the Orchidaceae and Fabaceae families. A high proportion of orchid species are projected to have short-lived seeds (Hay *et al*. 2010; Merritt *et al*. 2014), and as such could be dried and stored in liquid nitrogen directly without *in vitro* methods (Popova *et al*. 2016). However, orchid seeds are unusual in that they are routinely germinated *in vitro*, whether asymbiotically or symbiotically (Koene *et al*. 2020; Pujasatria *et al*. 2020), and many germinate readily, although some species require modifications in the media or conditions (Zale *et al*. 2022). The family Orchidaceae consists of over 27,000 species, mostly from the tropics and subtropics, but fewer than 2,000 have been assessed for their conservation status. However, of those, 48% were found to be threatened at some level (IUCN 2023). Thus, there will likely be thousands of orchid taxa that will require *ex situ* conservation to ensure their continued survival and, therefore, *in vitro* methods will be required for recovering their seeds from banking. With *in vitro* work reported for less than 100 of the over 800 genera of orchids, there is much work needed to fill that gap.

With the Fabaceae, a family of over 700 genera, this study's previous analysis of the cryopreservation literature

Table 7. Exceptional species listed in the Global Working List of Exceptional Plants (Pence *et al.* 2022), which were found in 25 or more articles in the Web of Science literature search

Exceptional Species	Woodiness[a] and climate	Number of Articles	Family	Number of Identified Exceptional Species in Family	Number of Total Articles in Family	Percent of Total Articles on One Species	Exceptionality Factor	Number of threatened congeners[c]
Carica papaya	Woody, tropical	123	Caricaceae	1	131	94%	3	6
Elaeis guineensis	Woody, tropical	123	Arecaceae	37	460	27%	3	0
Coffea arabica	Woody, tropical	106	Rubiaceae	32	264	40%	3	76
Cocos nucifera	Woody, tropical	79	Arecaceae	37	460	17%	2	0
Hevea brasiliensis	Woody, tropical	78	Euphorbiaceae	2	287	27%	2	9
Citrus sinensis	Woody[b], tropical	67	Rutaceae	35	369	18%	3	4
Theobroma cacao	Woody, tropical	67	Malvaceae	12	348	19%	2	1
Coffea canephora	Woody, tropical	56	Rubiaceae	32	264	21%	3	76
Quercus robur	Woody, temperate	51	Fagaceae	24	275	19%	2	161
Passiflora edulis	Woody[b], tropical	48	Passifloraceae	1	77	62%	3	0
Mangifera indica	Woody, tropical	47	Anacardiaceae	10	146	32%	2	25
Quercus suber	Woody, Mediterranean	46	Fagaceae	24	275	17%	3	161
Araucaria angustifolia	Woody, subtropical/temperate	45	Araucariaceae	10	51	88%	2	7
Castanea sativa	Woody, temperate	45	Fagaceae	24	275	16%	2	0
Citrus limon	Woody[b], tropical	45	Rutaceae	35	369	12%	3	4
Musa acuminata	Herbaceous, tropical	43	Musaceae	2	401	11%	3	8
Persea americana	Woody, subtropical	38	Lauraceae	24	75	51%	2	42
Dimocarpus longan	Woody, tropical	37	Sapindaceae	23	130	28%	2	2
Azadirachta indica	Woody, tropical	35	Meliaceae	25	108	32%	2	0
Corylus avellana	Woody, temperate	33	Betulaceae	3	129	26%	3	2

[a]From the BGCI GlobalTreeSearch database (BGCI 2023)

[b]Not in the BGCI GlobalTreeSearch database (BGCI 2023), therefore added manually

[c]From Pence *et al.* 2022, Supplemental Data, Table B5

yielded articles on only five of the 25 Fabaceae exceptional genera, and only one of those five genera was predominantly woody (*Inga*) (Table 4., Pence and Bruns 2022). However, with the *in vitro* literature, there were 12 Fabaceae genera in common between the literature and exceptional plants, and of those, five were predominantly woody genera. This will be important, as 75% of the species identified as exceptional have been assessed for woodiness with 95% of those

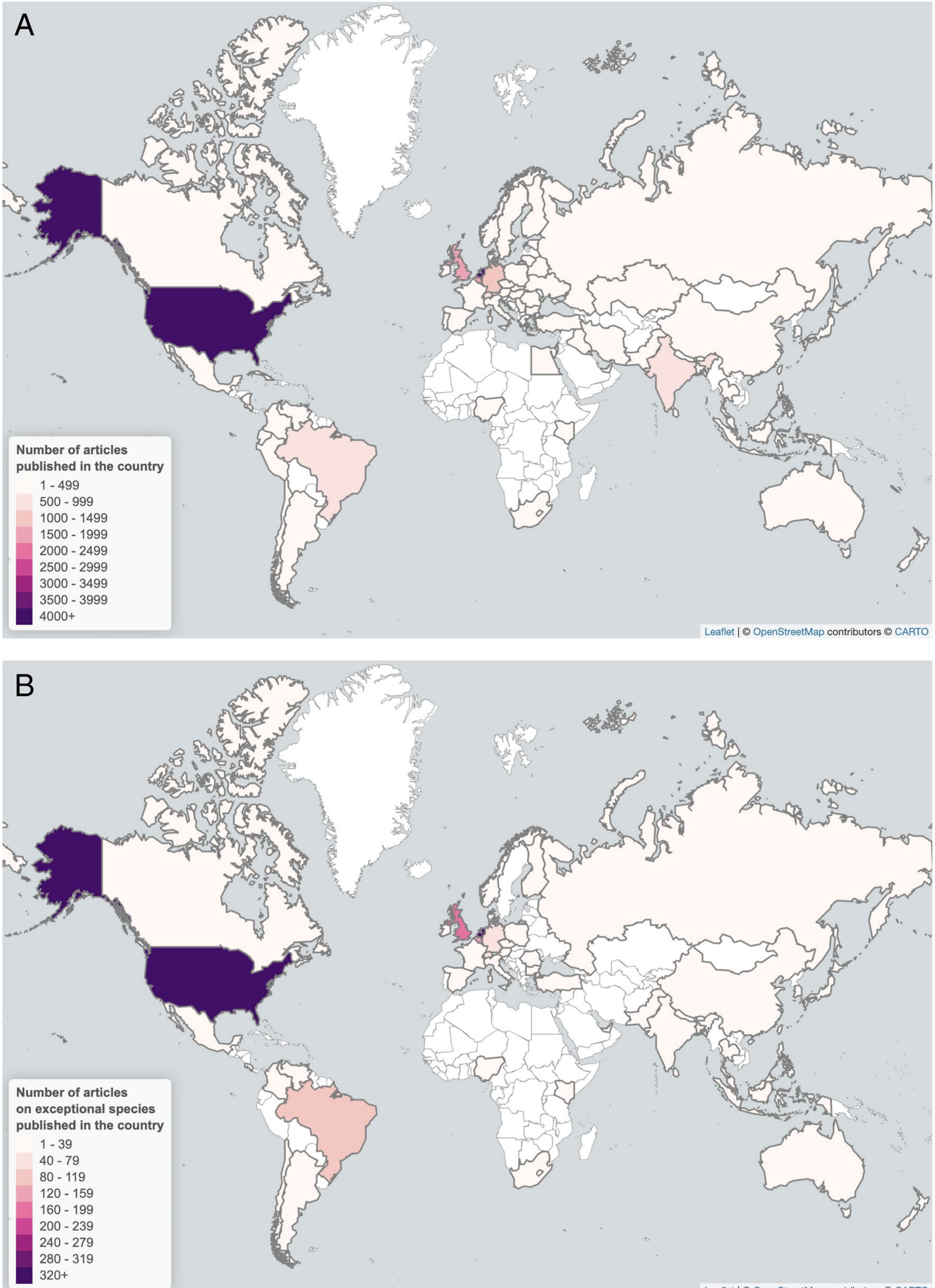

Figure 1. Heatmap of countries showing the number of *in vitro* articles published by a journal headquartered in that country. (*A*) All articles. (*B*) Articles on species in the Global Working List of Exceptional Plants (Pence *et al*. 2022). Note that the legend scales are different between the two maps.

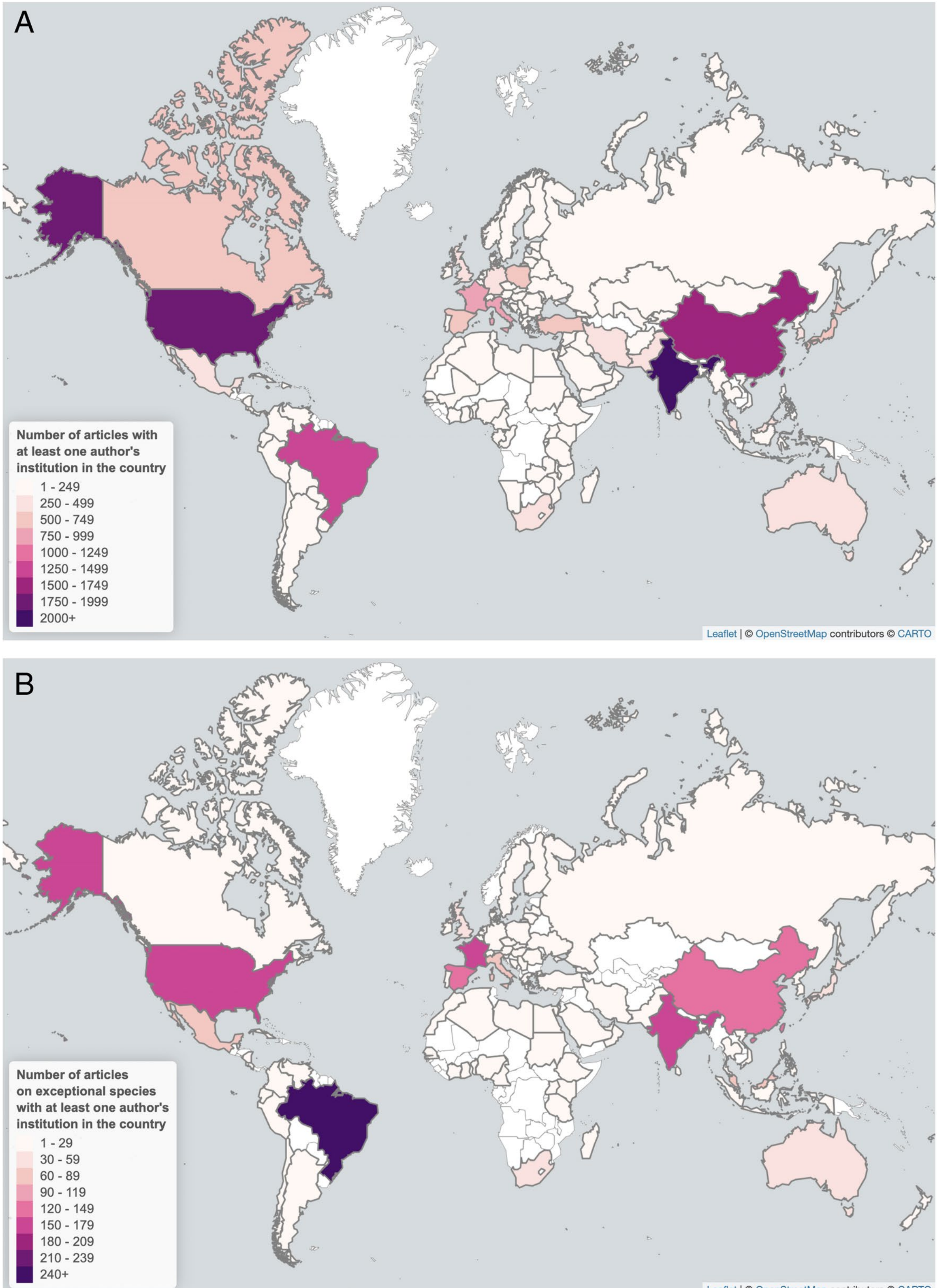

Figure 2. Heatmap of countries showing the number of *in vitro* articles produced by authors from institutions in those countries. Articles may have multiple authors representing multiple institutions/countries. (*A*) All articles. (*B*) Articles on species in the Global Working List of Exceptional Plants (Pence *et al*. 2022). Note that the legend scales are different between the two maps.

Table 8. Articles found with Google Scholar, which were not found in Web of Science searches for Dipterocarpaceae and Chrysobalanaceae

Type of Publication	References
Chrysobalanaceae	
Journal article, not in Web of Science	
African Journal of Biotechnology	Aboubacar and Sidikou 2018
Dipterocarpaceae	
Journal article, in Web of Science	
Physiologia Plantarum	Garello and Le Page-Degivry 1995
Pakistan Journal of Biological Sciences	Srisawat and Jongkraijak 2013
Sepilok Bulletin	Guanih *et al*. 2004
Plant Cell Tissue and Organ Culture	Scott *et al*. 1995; Linington 1991
In vitro Cellular and Developmental Biology–Plant	Shukla and Sharma 2017
Journal of Forest Science	Singh *et al*. 2014
Bulletin of the Forestry and Forest Products Research Institute	Ishii and Mohsin 1994
Journal article, not in Web of Science	
Journal of Medicinal Plants	Borpuzari and Kachari 2022
Jurnal Pemuliaan Tanaman Hutan	Yelnititis 2013
Bulletin of the Tokyo University of Forestry	Vaario 1996
Resource	Radzuan 2006
Commonwealth Forestry Review	Pinso and Moura-Costa 1993
Transactions of the British Mycological Society	Louis and Scott 1987
Journal of the Japanese Forestry Society	Vaario *et al*. 1993; Vaario *et al*. 1995
Proceedings	Gunasekara *et al*. 1988
	Jayanthi and Krishnapillay 2000
	Kandasamy *et al*. 2006
	Moura-Costa and Lundoh 1992
	Nakamura *et al*. 2000
	Normah and Aziah 2003
	Sakai and Yamamoto 1992
Book Section	Nakamura 2006
Institutional report	Muralidharan 2001
	Pollisco 1995
Thesis	Ee 2005

assessed being woody (Pence *et al*. 2022), and it is predicted that a high percentage of exceptional species will be woody, tropical species (Tweddle *et al*. 2003; Wyse and Dickie 2017). While a number of economically important tropical trees have been grown *in vitro* (Pijut *et al*. 2012), others have been particularly challenging *in vitro* and account for many of the species considered recalcitrant to tissue culture (McCown 2000). The 14 families with at least one known exceptional species not represented by any literature in this search were also mostly woody, native to the tropics, found in the Southern hemisphere, or were marine species. These areas contain high levels of biodiversity (Corlett 2016; Raven *et al*. 2020), are proportionately less studied than other areas (Westwood *et al*. 2021), and are threatened by agriculture, logging, habitat loss, unsustainable harvesting, and climate change (BGCI 2021).

There has been *in vitro* work with a number of economically important tropical woody species (Pijut *et al*. 2012), and this was also reflected in the current study. The 20 exceptional species with more than 25 articles each were all commercially important woody species and mostly tropical, providing a body of work that could help guide protocol development for the conservation of the many wild, threatened woody species projected to be exceptional. Half of the 20 are exceptional because of desiccation-sensitive seeds, and when this trait is found in one species, there is a high likelihood that it will be found in congeners as well (Wyse and Dickie 2017). The 343 threatened congeners of this group of species should be prioritized to determine how easily the well-developed protocols for these economically important species can be transferred to their wild relatives.

While this study found several areas where work has been done that will likely be useful for exceptional species conservation, it also revealed significant gaps in the current knowledge of *in vitro* culture. One particularly important family that had little *in vitro* research in the Web of Science

Table 9. Articles found with Google Scholar for genera that had no Web of Science articles identified and six or more species in the Global Working List of Exceptional Plants (Pence *et al.* 2022)

Type of Publication	References
Clermontia	
Dissertation	Koob 1996
Cyanea	
Journal article, in Web of Science	
Biological Conservation	Werden *et al.* 2020
Journal article, not in Web of Science	
Biodiversity	Murch 2004
Combined Proceedings of the International Plant Propagators' Society	Sugii *et al.* 2003
Dissertation	Koob 1996
Book Section	Sugii and Lamoureux 2004
Cyrtandra	
Conference Abstract	Philpott and Pence 2019
Melicope	
Journal article, in Web of Science	
In Vitro Cellular and Developmental Biology–Plant	Sugii 2011
Journal article, not in Web of Science	
International Journal of Pharma and Biological Science	Zuraida *et al.* 2014
Natural Science	Rahman *et al.* 2015
Thesis	Kikuchi 2008
Abstract	Philpott and Pence 2019
Rhizophora	
Journal article, not in Web of Science	
Life Sciences	I'anatushshoimah *et al.* 2020
Developmental Genetics	Robichaud *et al.* 1979

database is the Dipterocarpaceae, the family with the most species on the List of Exceptional Plants. Dipterocarps are the dominant trees in many Southeast Asian forests (Brearly *et al.* 2016). Because they are valued for timber, they are being logged at a high rate while their habitat is being lost to agriculture (Bartholomew *et al.* 2021). It is thought that a high percentage of dipterocarps, including species in the genera *Hopea* and *Shorea,* are exceptional, and this group should be prioritized for research.

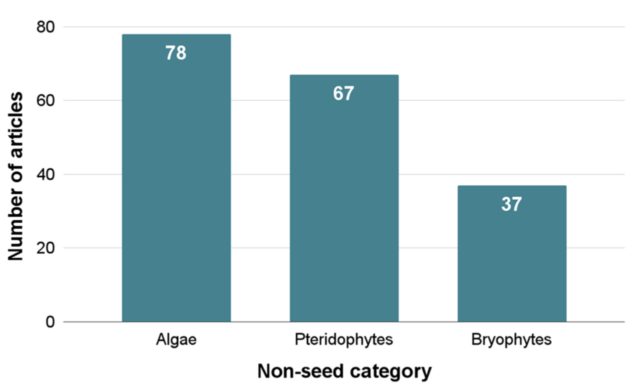

Figure 3. Number of Web of Science articles identified for each category of non-seed plants, and algae. No articles were identified for fungi.

Because of their importance and their low representation in the Web of Science literature, several families and genera were investigated further using the Google Scholar database. While only four articles on dipterocarps were captured in the original Web of Science search, which was not specifically targeted at Dipterocarpaceae, 26 additional articles were found in Google Scholar using the Web of Science search terms plus "Dipterocarpaceae". Using this targeted search did not retrieve additional articles in the Web of Science. The Google Scholar search retrieved a significant amount of gray literature in the form of reports, proceedings, and journals with a lower impact factor, primarily from Asia, where the family is native. However, there was little additional literature found through Google Scholar in searches for *in vitro* work with Zosteraceae and Chrysobalanaceae, two smaller families. When similar searches were done for the six genera lacking any literature in the Web of Science, similar types of literature were captured. This suggests that, although the widely used Web of Science has broad coverage of better-known journals and many features that facilitate its use and analysis, supplementing it with other sources may be needed for a thorough review for some families, particularly high-profile families like the Dipterocarpaceae.

Table 10. Families and genera of bryophytes, pteridophytes, and algae with two or more *in vitro* articles captured in the Web of Science search. The total number of families or genera with *in vitro* articles is given in parentheses

Bryophytes		Pteridophytes		Algae	
Top families (17 total)	Top genera (25 total)	Top families (14 total)	Top genera (31 total)	Top families (20 total)	Top genera (30 total)
Ditrichaceae	*Ditrichum*	Aspleniaceae	*Adiantum*	Euglenaceae	*Eucheuma*
Funariaceae	*Marchantia*	Cyatheaceae	*Asplenium*	Fucaceae	*Euglena*
Marchantiaceae	*Physcomitrella*	Lycopodiaceae	*Ceratopteris*	Gelidiaceae	*Fucus*
Pottiaceae	*Splachnum*	Osmundaceae	*Cyathea*	Gracilariaceae	*Gelidium*
Splachnaceae		Polypodiaceae	*Huperzia*	Solieriaceae	*Gracilaria*
		Pteridaceae	*Nephrolepis*	Volvocaceae	*Kappaphycus*
			Osmunda		*Volvox*
			Platycerium		
			Pteris		

Just as the Web of Science search has limitations, the List of Exceptional Plants is also not complete. It represents 2% or less of the projected number of exceptional plants and is considered a working list that will be periodically updated (Pence *et al*. 2022). However, while small in percentage, it is based on an evaluation of over 20,000 species. It confirms work by others that a significant group of exceptional species will be tropical trees (Tweddle *et al*. 2003) and can help in developing strategies and setting priorities, which can then be modified as the list evolves in the future.

In the course of these searches, information on non-seed plants was captured, along with that for seed plants. While the spores of ferns and bryophytes might be conserved by simple storage at low temperatures (Ballesteros *et al*. 2012), *in vitro* propagation of sporophytes and gametophytes of ferns and bryophytes, as well as of algae, can be useful in multiplying tissues when spores are few or absent, and these *in vitro*-grown tissues can then be conserved in liquid nitrogen (Day *et al*. 1999; Ballesteros and Pence 2018; Visch *et al*. 2019). While a focused search for non-seed groups might yield additional results, the searches done for this study revealed that at least some *in vitro* research has been done on a wide range of taxa within these groups.

While the major impetus for this study was to evaluate research on *in vitro* methods that support cryobiotechnology, the same *in vitro* methods can be valuable tools for propagating threatened species for restoration. For species with few seeds or for which conventional horticultural propagation is not successful, *in vitro* methods can and have been used for producing plants for restoring populations in the wild (Touchell *et al*. 1992; Skopec *et al*. 2017). While such projects depend on many factors, such as when funds, land, and labor are available, having predictable methods for producing healthy plants at low cost can be critical when restoration is needed to maintain a species in the wild.

A critical question for the conservation of exceptional species is how to expand cryobanking to a scale similar to that of seed banking (Bettoni *et al*. 2021; Zhang *et al*. 2023). Thus far, cryobanking tissues of threatened exceptional plants has been limited largely by the time and resources needed for protocol development for individual species. A significant part of that time is often spent developing the *in vitro* methods required for providing tissues for and/or recovering tissues after cryopreservation. While there is a large body of literature on *in vitro* culture of plants, the current study has shown that it only scratches the surface of the wide diversity of species that will need *in vitro* methods. Both technical advances and scientific insights are needed to overcome this hurdle. For example, methods for producing *in vitro* tissues quickly, such as generating buds from leaves, which are easy to prepare for cryopreservation, could help reduce the overall time needed for cryobanking (Espasandin *et al*. 2019; Wang *et al*. 2021). However, such protocols are only available for a relatively small number of species. Similarly, *in vitro* technologies for recovering and regrowing tissues cryopreserved directly from the source plant could eliminate the need to initiate and grow cultures before banking. This has been demonstrated in only a few cases thus far (for example, Kim *et al*. 2012; Pawlowska and Szewczyk-Taranek 2014; Volk *et al*. 2017; Bettoni *et al*. 2019) but would greatly reduce the time and cost and increase efficiency, if possible on a wider scale. A deeper understanding of the physiology of *in vitro* culture, including culture initiation, tissue stress and browning, plant recovery from *in vitro* tissues and acclimatization, and the relationship of these to the natural growth and adaptations of species, could all help reveal *in vitro* response patterns and add predictive capacity to *in vitro* culture. There is a significant need for comparative studies of *in vitro* physiology and development to work toward this goal, and such progress can grow from the foundation that has been laid from decades of *in vitro* work with hundreds of plant species, as represented in the present Web of Science search. Understanding the growth and adaptations of a wider range of species to *in vitro* culture could lead to greater efficiencies that are important for scaling up the efforts needed to meet the challenge of exceptional plant conservation.

Conclusions

This study revealed that, while there has been a large body of *in vitro* research on plants, much of that work has been focused on food and other commercially important species, and there are significant taxonomic gaps when the literature is compared with the List of Exceptional Plants. One particular gap is with tropical woody species, a highly diverse and threatened group with a high percentage of exceptional species. A broader focus for *in vitro* research is needed, encompassing a wider range of taxa, to meet the significant challenge of the long-term conservation of exceptional plants.

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Data availability Restrictions apply to the availability of these data. Data were obtained from Web of Science and are available from the authors with the permission of Web of Science. Supporting data and scripts used for analysis are publicly available at https://github.com/eb-bruns/WoS_wordsearch_invitro.

Declarations

Conflict of interest The authors declare no competing interests.

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