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# An analysis of the literature of ex situ germplasm preservation

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# An analysis of the literature of *ex situ* germplasm preservation<sup>1</sup>

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## Summary

Few scientific journals or other serial publications emphasize reports of research on germplasm conservation, making it difficult for scientists and research librarians to identify key publications in this discipline. Citation analysis was used to determine most-cited works on the storage of seeds, pollen and plant tissue cultures. Two hundred research articles published during the 1980s were used in this study. They contained 3674 citations of published works, with 2926 of journal articles or other serials, 399 of book chapters and 349 of complete books and theses. The 100 most-cited works accounted for 22% of all citations. Lists of most-cited works are included. Citations were concentrated in particular journals. A core of 28 journals comprised half of all citations of serial publications. The most common disciplines covered by these 28 journals are horticulture, general botany, seed science, plant physiology, agronomy and general science.

## Introduction

There are very few scientific journals solely devoted to germplasm conservation, making it difficult for researchers scattered among the world's germplasm conservation facilities to locate pertinent research reports. Germplasm conservation is an emerging scientific discipline. But to the authors' knowledge, no abstracting service for, or objective analysis of, the literature of this discipline has been developed.

Many disciplines do have clearly identifiable journals that can be examined and ranked using comprehensive tools such as the Institute for Scientific Information Data Base or Science Citation Index Journal Citation Reports. Typical reports of such rankings include analyses of agricultural and botanical journals (Garfield, 1977, 1981a, b), a series of papers covering physiology, pharmacology and microbiology journals (Sengupta, 1973, 1974a, b), and an analysis of chemistry publications in India (Singh, 1974).

These reports of rankings rely on a technique known as citation analysis (Garfield, 1972). Citation analysis is based on the relationship between publications and items cited in those publications. By carefully selecting publications for analysis and then compiling lists of cited items, one can identify the

most-cited serial publications and individual works important for a particular discipline. This information can be used by scientists and research librarians to find key publications and to build appropriate research libraries.

There are definite limitations to the use of citation analysis. Analyses based on small samples or those that emphasize the works of few researchers may give misleading results. The number of items chosen for analysis should be large enough to represent the body of literature to be studied. And the works of a diverse group of researchers should be used to avoid individual biases in making citations.

Considering these limitations, we began a project in 1989 to analyze citations from the scientific literature of germplasm conservation. We had two goals for this project. First, we hoped to identify a core group of serial publications that can be considered essential for research libraries at genebanks and that prospective authors could use to identify the most widely read journals. Second, we hoped to produce a list of the most-often cited works in germplasm conservation.

The literature of germplasm conservation includes many topics and draws on a number of disciplines. So we have divided this body of literature into three broad topics: *ex situ* germplasm preservation; acquisition and exploration; and germplasm regeneration methods. This paper reports on the analysis of research papers on *ex situ* germplasm preservation. The remaining topics will be examined in future reports. For the purposes of this project, the topic of *ex situ* germplasm preservation includes research on the storage of seeds (both orthodox and recalcitrant), pollen and tissue cultures, as well as theoretical studies of longevity during storage.

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## Materials and Methods

Two hundred research reports on *ex situ* germplasm preservation were chosen from scientific journals published between 1980 and 1989. These reports were selected primarily from four sources: the authors' personal literature files; *Recalcitrant Seeds: A Bibliography 1979-87* (Pritchard, 1988); *A Bibliography of Crop Genetic Resources* (Hawkes *et al.*, 1983); and an unpublished bibliography of publications on the cryogenic storage of pollen (Connor, unpublished).

An attempt was made to sample, as evenly as possible, research on the storage of seeds (both orthodox and recalcitrant), pollen and tissue cultures. However, 53% of the articles reported on seed-storage research (Table 1). Also, international and non-US journals were favoured to counteract an unavoidable bias towards US publications in the authors' personal files. Most articles in this study were written by researchers outside of the US (Table 1).

Sengupta (1973, 1974a, b) ranked the most-cited journals of physiology, pharmacology and microbiology based on an analysis of citations found solely in review articles. However, Broadus (1977) maintained that citations in review articles do not reflect the degree to which articles actually contribute to the body of scientific knowledge. He wrote that "the 'true' citation analysis deals with works cited as actually having been used in the preparation of, or having otherwise contributed to, the source paper". Accordingly, the articles chosen for this analysis emphasize reports of original research and are supplemented by only a few specialized, critical reviews.

Table 1. Characteristics of the 200 articles analyzed

Topic	US authored	Non-US authored	Total
Orthodox seeds	20	39	59
Recalcitrant seeds	6	41	47
Tissue culture	16	29	45
Pollen	19	14	33
Theoretical and mixed	3	13	16
Total	64	136	200

The 200 selected research reports contain 3674 citations of published works. These citations were tabulated and sorted using dBase III Plus software\* and were manually examined to identify the individual works and particular serials most often cited. A list of the 200 articles examined can be obtained upon request.

## Results and Discussion

The 3674 citations include 2926 of journal articles or other serial publications, 399 of book chapters and 349 of complete books and theses. The 42 most-cited works are listed in Table 2. This list accounts for 13% of all citations and expanding it to the 100 most-cited works accounts for 22% of all citations. Two works listed in Table 2 have exceptionally high citation frequencies. These are the important tissue culture medium report of Murashige and Skoog (1962) and

\* Mention of commercial products is not to be construed as an endorsement of any product by USDA-ARS or its cooperators.

Table 2. The most-cited works

Times cited	Complete citation	Times cited	Complete citation
30	Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. <i>Physiol. Plant.</i> 15:473-497.	13	Sakai, A., M. Yamakawa, D. Sakata, T. Harada and T. Yakuwa. 1978. Development of whole plant from an excised strawberry runner apex frozen to -196°C. <i>Low Temp. Sci. Ser. B. Biol. Sci.</i> 36:31-38.
30	Roberts, E.H. 1973. Predicting the storage life of seeds. <i>Seed Sci. Technol.</i> 1:499-514.	13	Stanley, R.G. and H.F. Linskens. 1974. <i>Pollen: Biology, Biochemistry, Management.</i> Springer-Verlag, Berlin.
18	International Board for Plant Genetic Resources. 1976. Report of the IBPGR Working Group on Engineering, Design and Cost Aspects of Long-term Seed Storage Facilities. IBPGR, Rome.	12	Ellis, R.H. and E.H. Roberts. 1980. Improved equations for the prediction of seed longevity. <i>Ann. Bot. (London)</i> 45:13-30.
18	King, M.W. and E.H. Roberts. 1979. The Storage of Recalcitrant Seeds - Achievements and Possible Approaches. IBPGR, Rome.	12	Kartha, K.K., N.L. Leung and K. Pahl. 1980. Cryopreservation of strawberry meristems and mass propagation of plantlets. <i>J. Am. Soc. Hort. Sci.</i> 105:481-484.
16	Groat, B.W.W. and G.G. Henshaw. 1978. Freeze-preservation of potato shoot tip cultures. <i>Ann. Bot. (London)</i> 42:1227-1229.	12	Roberts, E.H. 1972. Storage environment and the control of viability. In: <i>Viability of Seeds</i> (E.H. Roberts, ed.), pp. 14-58. Chapman & Hall, London.
14	International Seed Testing Association. 1976. International rules for seed testing. <i>Seed Sci. Technol.</i> 4:3-49.	11	Barnabas, B. and E. Rajki. 1976. Storage of maize ( <i>Zea mays</i> L.) pollen at -196°C in liquid nitrogen. <i>Euphytica</i> 25:747-752.
14	Roberts, E.H. 1975. Problems for long term storage of seed and pollen for genetic resources conservation. In: <i>Crop Genetic Resources for Today and Tomorrow</i> (O.H. Frankel and J.G. Hawkes, eds.), pp. 269-296. Cambridge University Press, Cambridge.	11	Seibert, M. 1976. Shoot initiation from carnation apices frozen to -196°C. <i>Science</i> 191:1178-1179.
13	Kartha, K.K., N.L. Leung and O.L. Gamborg. 1979. Freeze-preservation of pea meristems in liquid nitrogen and subsequent plant regeneration. <i>Plant Sci. Lett.</i> 15:7-15.	9	Abdalla, F.H. and E.H. Roberts. 1968. The effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans and peas during storage. <i>Ann. Bot. (London)</i> 32:119-136.

Table 2. (continued)

Times cited	Complete citation	Times cited	Complete citation
9	Bajaj, Y.P.S. and J. Reinert. 1977. Cryobiology of plant cell cultures and establishment of gene banks. In: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture (J. Reinert and Y.P.S. Bajaj, eds.), pp. 757-789. Springer-Verlag, Berlin.	8	Barton, L.V. 1965. Viability of seeds of <i>Theobroma cacao</i> L. Contrib. Boyce Thompson Inst. 23:109-122.
9	Bajaj, Y.P.S. 1979. Technology and prospects of cryopreservation of germplasm. Euphytica 28:267-285.	8	Chin, H.F. and E.H. Roberts (eds.). 1980. Recalcitrant Crop Seeds. Tropical Press, Kuala Lumpur.
9	Barton, L.V. 1961. Seed Preservation and Longevity. Leonard Hill, London.	8	Collins, F.C., V. Lertmongkol and J.P. Jones. 1973. Pollen storage of certain agronomic species in liquid air. Crop Sci. 13:493-494.
9	Ellis, R.H. and E.H. Roberts. 1980. The influence of temperature and moisture on seed viability period in barley ( <i>Hordeum distichum</i> L.). Ann. Bot. (London) 45:31-37.	8	Ellis, R.H. and E.H. Roberts. 1980. Towards a rational basis for testing seed quality. In: Seed Production (P.D. Hebblethwaite, ed.), pp. 605-635. Butterworths, London.
9	Finkle, B.J. and J.M. Ulrich. 1979. Effects of cryoprotectants in combination on the survival of frozen sugarcane cells. Plant Physiol. (Bethesda) 63:598-604.	8	Ellis, R.H. and E.H. Roberts. 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol. 9:373-409.
9	Mullin, R.H. and D.E. Schlegel. 1976. Cold storage maintenance of strawberry meristem plantlets. HortScience 11:100-101.	8	Grout, B.W.W., R.J. Westcott and G.G. Henshaw. 1978. Survival of shoot meristems of tomato seedlings frozen in liquid nitrogen. Cryobiology 15:478-483.
9	Nath, J. and J.O. Anderson. 1975. Effect of freezing and freeze-drying on the viability and storage of <i>Lilium longiflorum</i> L. and <i>Zea mays</i> L. pollen. Cryobiology 12:81-88.	8	Roberts, E.H. 1960. The viability of cereal seed in relation to temperature and moisture. Ann. Bot. (London) 24:12-31.
9	Roberts, E.H. 1972. Cytological, genetical and metabolic changes associated with loss of viability. In: Viability of Seeds (E.H. Roberts, ed.), pp. 253-306. Chapman & Hall, London.	8	Roberts, E.H. 1973. Loss of viability: chromosomal and genetical aspects. Seed Sci. Technol. 1:515-527.
9	Roberts, E.H. and R.H. Ellis. 1977. Prediction of seed longevity at sub-zero temperatures and genetic resources conservation. Nature 268:431-433.	8	Sakai, A. and M. Noshiro. 1975. Some factors contributing to the survival of crop seeds cooled to the temperature of liquid nitrogen. In: Crop Genetic Resources for Today and Tomorrow (O.H. Frankel and J.G. Hawkes, eds.), pp. 317-326. Cambridge University Press, Cambridge.
9	Roberts, E.H. 1978. Mutations during storage. Acta Hort. (The Hague) 83:279-282.	8	Sakai, A. and Y. Nishiyama. 1978. Cryopreservation of winter vegetative buds of hardy fruit trees in liquid nitrogen. HortScience 13:225-227.
9	Seibert, M. and P.J. Wetherbee. 1977. Increased survival and differentiation of frozen herbaceous plant organ cultures through cold treatment. Plant Physiol. (Bethesda) 59:1043-1046.	8	Uemura, A. and A. Sakai. 1980. Survival of carnation ( <i>Dianthus caryophyllus</i> L.) shoot apices frozen to the temperature of liquid nitrogen. Plant Cell Physiol. 21:85-94.
9	Visser, T. 1955. Germination and storage of pollen. Meded. Landbouwhoges. Wageningen 55:1-68.	8	Villiers, T.A. 1974. Seed ageing: Chromosome stability and extended viability of seed stored fully imbibed. Plant Physiol. (Bethesda) 53:875-879.
9	Withers, L.A. 1979. Freeze preservation of somatic embryos and clonal plantlets of carrot ( <i>Daucus carota</i> L.). Plant Physiol. (Bethesda) 63:460-467.	8	Weatherhead, M.A., B.W.W. Grout and G.G. Henshaw. 1978. Advantages of storage of potato pollen in liquid nitrogen. Potato Res. 21:331-334.

the report by Roberts (1973) on mathematical models to predict the storage life of seeds.

Table 3 lists the 23 most-cited books and theses. This list was developed by combining citations of complete books with those of book chapters. To a certain extent, books are thus weighted by the frequency of citations of chapters. As with Table 2, two works stand out. These are *Crop Genetic Resources for Today and Tomorrow* edited by Frankel and Hawkes (1975), which contained nine cited chapters, including two that were highly cited (Table 2), and *Viability of Seeds* edited by Roberts (1972), which also contained nine cited chapters with two listed in Table 2.

For the purposes of identifying core journals, Table 4 lists the 28 most-cited serial publications and their places of publication. This list includes 50% of all serial citations. Most of the journals listed in Table 4 publish reports in one of six fields: horticulture, gen-

eral botany, seed science, plant physiology, agronomy and general science. Seven of the journals specialize in horticulture, and six in general botany.

Twelve of the 28 journals listed in Table 4 were not among the journals represented in the initial sample of 200 articles analyzed. This suggests that key journals consulted by researchers involved in *ex situ* germplasm preservation include a more diverse range of disciplines than those in which they publish. Conversely, it should be noted that three journals not listed in Table 4 were well represented (at least five articles each) in the initial sample. These journals are *Research Bulletin of the N.I. Vavilov Institute*, *Scientia Horticulturae* and *Tropical Agriculture*. Although these journals contain useful information, they may not be widely cited because of language barriers or limited distribution.

We did not begin this project with defined expectations that could be used to judge these results.

Table 3. The most-cited books and theses

Times cited	Complete citation
46	Frankel, O.H. and J.G. Hawkes. (eds.) 1975. <i>Crop Genetic Resources for Today and Tomorrow</i> . Cambridge University Press, Cambridge.
41	Roberts, E.H. (ed.) 1972. <i>Viability of Seeds</i> . Chapman & Hall, London.
19	Chin, H.F. and E.H. Roberts. (eds.) 1980. <i>Recalcitrant Crop Seeds</i> . Tropical Press, Kuala Lumpur.
18	International Board for Plant Genetic Resources. 1976. <i>Report of the IBPGR Working Group on Engineering, Design and Cost Aspects of Long-term Seed Storage Facilities</i> . IBPGR, Rome.
18	King, M.W. and E.H. Roberts. 1979. <i>The Storage of Recalcitrant Seeds - Achievements and Possible Approaches</i> . IBPGR, Rome.
17	Li, P.H. and A. Sakai. (eds.) 1978. <i>Plant Cold Hardiness and Freezing Stress: Mechanisms and Crop Implications</i> . Academic Press, New York.
16	Reinert, J. and Y.P.S. Bajaj. (eds.) 1977. <i>Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture</i> . Springer-Verlag, Berlin.
15	Stanley, R.G. and H.F. Linskens. 1974. <i>Pollen: Biology, Biochemistry, Management</i> . Springer-Verlag, Berlin.
14	Hebblethwaite, P.D. 1980. <i>Seed Production</i> . Butterworths, London.
13	Frankel, O.H. and E. Bennett. (eds.) 1970. <i>Genetic Resources in Plants - Their Exploration and Conservation</i> . Blackwell Scientific, Oxford.
12	Withers, L.A. 1980. <i>Tissue Culture Storage for Genetic Conservation</i> . IBPGR, Rome.
11	Kozłowski, T.T. (ed.) 1972. <i>Seed Biology</i> . Academic Press, New York.
11	Akihama, T. and K. Nakajama. (eds.) 1978. <i>Long-term Preservation of Favourable Germplasm in Arboreal Crops</i> . Japanese Ministry of Agriculture and Forestry, Fujimoto.
10	Kartha, K.K. (ed.) 1985. <i>Cryopreservation of Plant Cells and Organs</i> . CRC Press, Boca Raton.
9	Barton, L.V. 1961. <i>Seed Preservation and Longevity</i> . Leonard Hill, London.
9	Thorpe, T.A. (ed.) 1978. <i>Frontiers of Plant Tissue Culture 1978</i> . International Association for Plant Tissue Culture, Calgary.
9	Meryman, H.T. (ed.) 1966. <i>Cryobiology</i> . Academic Press, New York.
8	Heydecker, W. (ed.) 1972. <i>Seed Ecology</i> . Butterworths, London.
8	Barz, W., E. Reinhard and M.H. Zenk. (eds.) 1977. <i>Plant Tissue Culture and its Bio-technological Application</i> . Springer-Verlag, Berlin.
7	Khan, A.A. (ed.) 1982. <i>The Physiology and Biochemistry of Seed Development, Dormancy, and Germination</i> . Elsevier Biomedical, Amsterdam.
7	Murata, M. 1979. <i>Genetic Changes Induced by Artificial Seed Ageing in Barley</i> . PhD Thesis. Colorado State University, Fort Collins.
6	Cromarty, A.S., R.H. Ellis and E.H. Roberts. 1982. <i>The Design of Seed Storage Facilities for Genetic Conservation</i> . IBPGR, Rome.
6	Ashwood-Smith, M.J. and J. Farrant. (eds.) 1980. <i>Low Temperature Preservation in Medicine and Biology</i> . Pitman, London.

Table 4. The most-cited serial publications

Times cited	Title and place of publication
183	Seed Science and Technology, Zurich, Switzerland
153	Annals of Botany, London, Great Britain
132	Plant Physiology, Rockville, MD, USA
115	HortScience, Alexandria, VA, USA
73	Crop Science, Madison, WI, USA
66	Physiologia Plantarum, Lund, Sweden
65	Cryobiology, New York, NY, USA
56	Journal of the American Society for Horticultural Science, Alexandria, VA, USA
52	Plant Science, Amsterdam, Netherlands
51	Nature, London, Great Britain
51	Proceedings of the American Society for Horticultural Science, St. Joseph, MI, USA
45	Euphytica, Dordrecht, Netherlands
35	American Journal of Botany, Columbus, OH, USA
34	Journal of Experimental Botany, Oxford, Great Britain
34	Journal of Horticultural Science, Ashford, Great Britain
31	Arboretum Kórnickie, Warsaw, Poland
29	Canadian Journal of Botany, Ottawa, ON, Canada
27	Potato Research, Wageningen, Netherlands
27	Proceedings of the International Seed Testing Association, Ås, Norway
24	Phytopathology, St. Paul, MN, USA
23	Agronomy Journal, Madison, WI, USA
23	Annual Review of Plant Physiology, Palo Alto, CA, USA
23	FAO/IBPGR Plant Genetic Resources Newsletter, Rome, Italy
23	Proceedings of the Association of Official Seed Analysts, Lansing, MI, USA
22	Contributions from Boyce Thompson Institute, Yonkers, NY, USA
22	Science, Washington, DC, USA
21	Planta, Heidelberg, Federal Republic of Germany
20	Acta Horticulturae, Wageningen, Netherlands

However, the results do seem consistent with our *a priori* understanding of this body of literature. In general, journals listed in Table 4 and books listed in Table 3 are ones we have consulted in research, although some of the books are becoming outdated.

Librarians associated with genebanks can evaluate their holdings guided by our findings and perhaps identify potential acquisitions for their libraries. Our findings, however, are based on a historical record of literature use, albeit recent; and it may be that some of the key research publications of the 1980s will become outmoded. Moreover, like four of the 28 serials listed in Table 4, serial publications may be discontinued or change focus. For students of the history of *ex situ* germplasm preservation, our findings may also be interesting. The works cited in Table 2 serve as a foundation for research in the 1980s, and a citation analysis of these older works can lead back to the beginnings of this research topic.

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## Résumé

### *Analyse de la littérature sur la préservation du matériel génétique ex situ*

Comme peu de journaux scientifiques ou autres périodiques mettent en évidence les rapports de recherche sur la conservation du matériel génétique, les scientifiques et les bibliothécaires de centres de recherche ont du mal à identifier les principales publications dans cette discipline. Pour identifier les travaux les plus souvent cités sur le stockage des semences, le pollen et les cultures tissulaires de plantes, on a analysé des citations révélées dans 200 articles sur des travaux de recherche publiés au cours des années 80. Ces articles contenaient 3674 renvois à des travaux publiés, dont 2926 à des articles de journaux ou autres publications, 319 à des chapitres d'ouvrages et 349 à des ouvrages et à des thèses proprement dits. Les 100 ouvrages les plus cités font l'objet de 22 pour cent de toutes les citations. La liste des ouvrages les plus souvent cités est incluse. Les citations proviennent d'un nombre restreint de journaux. La moitié de toutes les citations renvoyant à des périodiques figurent dans un noyau de 28 journaux. Les principales disciplines couvertes par ces 28 journaux sont l'horticulture, la botanique générale, la science des semences, la physiologie des plantes, l'agronomie et la science en général.

## Resumen

### *Análisis de la bibliografía sobre la conservación de germoplasma ex situ*

Son pocas las revistas científicas u otras publicaciones periódicas que conceden suficiente importancia a los informes sobre las investigaciones relativas a la conservación de germoplasma, por lo que los científicos y los investigadores bibliográficos encuentran dificultades para identificar publicaciones que se ocupen directamente de esta disciplina. Para determinar los trabajos más citados sobre el almacenamiento de semillas, polen y cultivos de tejidos vegetales, se recurrió al análisis de las citas bibliográficas. El presente estudio se basó en 200 artículos sobre investigación publicados durante los años ochenta. En ellos aparecían 3674 citas de publicaciones, de las cuales 2926 correspondían a artículos de diarios o de otras publicaciones periódicas, 399 a capítulos de libros y 349 a libros completos y tesis. El 22 por ciento de todas las citas correspondía a los 100 trabajos más citados. En el presente artículo se incluye una lista de los trabajos más citados. Las citas se concentraban en determinados diarios. La mitad de todas las citas de las publicaciones periódicas aparecían en 28 diarios. Las disciplinas tratadas con mayor frecuencia son la horticultura, la botánica general, la ciencia de las semillas, la fisiología vegetal, la agronomía y las ciencias en general.