出國報告（出國類別：實習）

參加「植物遺傳資源體外與超低溫冷凍保存方法」訓練課程研習報告

服務機關：行政院農業委員會種苗改良繁殖場
姓名職稱：林如玲 助理研究員
派赴國家/地區：印度新德里
出國期間：108 年 11 月 4 日至 11 月 20 日
報告日期：108 年 1 月 9 日

摘要

本場為我國種苗研究的專責機構，核心業務包括有種子、種苗供應及育種技術研發等，而種原的保存是生產和育種的基石，特別是目前本場量產供應有馬鈴薯、草莓、芋頭、綠竹等無性繁殖作物，這些作物無法以傳統種原庫的方式進行保存，亟需新的保存方法，以克服田間定植保存因氣候變遷所造成的氣候不穩定及病蟲害等問題，及組織培養試管保存所產生的遺傳變異。根據 FAOSTAT 統計，營養繁殖型及異儲型種子作物的產量每年約有 10 億噸，產值高達 1000 億美元，因此確保這些作物的種原保存，對未來我國發展韌性農業及確保糧食安全影響甚鉅。本次參加「第 8 屆植物遺傳資源體外和冷凍保存方法」國際訓練課程，由印度國家植物遺傳資源局（NBPGR）、印度國際生物多樣性辦公室及亞太農業研究機構聯盟(APAARI)共同舉辦，藉由該訓練希望能獲得冷凍保存技術所需的基本知識、提升組織培養技術在保存與管理植物遺傳資源的應用，以及瞭解分子技術如何運用在種原管理。訓練期間與該領域首屈一指的國際學者及亞、非各國參與訓練的學員積極互動、建立友誼，期許未來在熱帶林木及熱帶作物種原保存方法開發有進一步的技術交流，以為新南向區域的種原保存活動提供新的助力。

目次

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本場為我國種苗研究的專責機構，核心業務包括有種子、種苗供應及育種技術研發等，而種原的保存是生產和育種的基石，特別是目前本場量產供應有馬鈴薯、草莓、芋頭、綠竹等無性繁殖作物，這些作物無法以傳統種原庫的方式進行保存，亟需新的保存方法，以克服田間定植保存因氣候變遷所造成的氣候不穩定及病蟲害等問題，及組織培養試管保存時所產生的遺傳變異。根據 FAOSTAT 統計，營養繁殖型及異儲型種子作物的產量每年約有 10 億噸，產值高達 1000 億美元，因此確保這些作物的種原保存，對未來我國產業發展韌性農業及確保糧食安全等農業政策影響甚鉅。我國為亞太農業研究機構聯盟(Asia-Pacific Association of Agricultural Research Institutions, APAARI）的會員，該組織針對國際農業重要議題舉辦訓練課程，進行知識與經驗分享，本次為「植物遺傳資源體外與超低溫冷凍保存方法」國際訓練第 8 次舉辦，凸顯生物多樣性公約賦予資源國對於境內生物遺傳資源的所有權後，各國對境內遺傳資源保護的重視。本次參加此訓練課程希望能獲得冷凍保存技術所需的基本知識、提升組織培養技術在保存與管理植物遺傳資源的應用、以及瞭解分子技術如何運用在種原管理，同時期許有新的機會對熱帶異儲型及無性繁殖作物進行長期保存方法的開發，並與本次參與的亞非各國能有進一步的技術交流。

貳、過程

一、課程訓練概況

本次訓練是由印度國家植物遺傳資源局（ICAR-National Bureau of Plant Genetic Resources, NBPGR）和印度國際生物多樣性辦公室(Bioversity International , Bl)共同籌辦。NBPGR 是印度農業研究所(ICAR)之下負責植物遺傳資源管理的首要研究所，自 2006 年以來，NBPGR 被指定為卓越中心（CoE）接受有關體外保存和冷凍保存的國際培訓計劃，目前也是世界第 2 大的種原保存中心。今年，該培訓計畫與泰國曼谷亞太農業研究機構聯盟（APAARI）旗下的亞太農業生物技術和生物資源聯盟（APCoAB）計劃共同合作。

本次訓練地點位於印度新德里市區的 Pusa 園區，共計有 22 位學員參與訓練，分別來自 13 個國家，包括: 阿爾及利亞、孟加拉國、台灣、斐濟、印度、哈薩克斯坦、馬達加斯加、薩摩亞、塞內加爾、巴布亞新幾內亞、菲律賓、烏克蘭、烏茲別克斯坦。學員來自多重領域，包括大學教授、研究機構人員、各國植物遺傳資源管理人員、組織培養實驗室主管及學生等。講師主要為印度農業研究所及國家植物遺傳資源局的研究人員，並邀請植物遺傳資源保存領域多位著名學者，包括英國國家植物園邱園的 Prof. Huge Pritchard，其曾擔任「種子保存系」及「千禧年種子

銀行計劃」(Millennium Seed Bank Project)的研究主管；比利時魯汶大學 Bioversity International 的 Dr Bart Panis，該中心目前收藏了 1500 多種野生和食用香蕉品種及 V-cryoplate & D-cryoplate 技術發明人 Dr Takao Niino (孝男新野) 進行講課與實作指導。

訓練方式主要包括授課及實作，授課內容包括下面幾個主軸: 1.植物遺傳資源的保存方式、資訊管理以及知識管理 2.超低溫冷凍保存技術的基本原理、技術與應用、V-cryoplate 和 D-cryoplate 新技術的操作應用 3.全球植物遺傳資源保存庫的經驗及超低溫冷凍技術的展望等。實作課程從溶液及培養基配置、組織培養操作、超低溫冷凍技巧、不同植物組織來源的操作方式、利用分子生物學進行種原遺傳變異分析及種原病原檢測等進行一系列操演，希望學員能熟悉成立種原庫必備的各項操作，於未來能利用此保存技術對本國相關作物植物資源開發有效的保存方案。

二、研習行程

日期	研習內容與活動行程
11 月 4 日(一)	由桃園國際機場啟程，經香港國際機場轉機，飛往印度新德里，NBPGR 派員接機至住宿旅館
11 月 5 日(二)	報到、領取資料、進行開業典禮、並由學員自我介紹，留影合照後進入正式研習課程。 課程 1.亞太農業生物科技聯盟(APCoAB) 在促進亞太地區生物技術應用和能力發展所扮演之角色 課程 2.介紹印度國家植物遺傳資源署(NBPGR)如何進行植物遺傳資源的體外與超低溫冷凍保存 研習前評量測試
11 月 6 日(三)	課程 3.蘭花遺傳資源微繁殖和冷凍保存-泰國經驗 實習 1.超低溫冷凍保存溶液及培養基配製 實習 2.單子葉和雙子葉植物芽尖及分生組織的分離 課程 4.植物遺傳資源的資訊管理
11 月 7 日(四)	課程 5. 體外冷凍保存技術介紹 課程 6. 冷凍保存的原理和超低溫冷凍保存庫實務 實習 3. 冷凍保存前非耐貯型水果/種子處理 實習 4. 使用玻璃質化技術進行冷凍保存 課程 7. 印度植物遺傳資源管理概述
11 月 8 日(五)	課程 8. V-cryoplat 及 D-cryoplate 超低溫冷凍保存法之發展

	實習 5. V-cryoplat 及 D-cryoplate 超低溫冷凍保存法實作
11 月 9 日(六)	課程 9. 休眠芽冷凍保存理論與實務 課程 10.溫帶果樹的體外保存和冷凍保存 實習 6. 休眠芽的超低溫冷凍保存
11 月 10 日(日)	假日-安排德里市區觀光-自費參訪胡馬雍陵(Humayun’s Tomb)、古達明納塔(Qutab Minar)印度門(India gate)及戰士紀念碑等
11 月 11 日(一)	課程 11.種子超低溫生物技術基本概論 實習 7. 非耐貯型種子的多種冷凍保存法 參觀 NBPGR 的種子庫及超低溫冷凍保存庫
11 月 12 日(二)	課程 12.種子超低溫保存技術的成就與展望 課程 13.受脅迫生態下的植物種原冷凍保存-印度經驗 實習 8.利用藻膠包埋脫水法進行超低溫冷凍保存之實作 學員經驗分享報告：6 位
11 月 13 日(三)	參觀 NBPGR 栽培植物標本館 課程 14.花粉冷凍保存-植物育種的輔助工具 實習 9.香蕉不同組織部位的種原保存方式實作 學員經驗分享報告：7 位
11 月 14 日(四)	課程 15.塊莖作物的體外保存和超低溫冷凍保存 實習 10.花粉冷凍保存實作 參觀 NBPGR 的體外保存種原基因庫 學員經驗分享報告：2 位
11 月 15 日(五)	課程 16.體外保存和超低溫冷凍保存種質的遺傳穩定性研究 課程 17.作物遺傳資源的國際保護和利用現況 實習 11.大蒜超低溫冷凍保存實作 學員經驗分享報告：1 位
11 月 16 日(六)	課程 18.體外基因庫和低溫冷凍保存庫種質的健康檢測 實習 12.植物 DNA 萃取及電泳 實習 13.SSR 分子標誌 PCR 分析 學員經驗分享報告：5 位
11 月 17 日(日)	假日-安排阿格拉觀光-自費參訪泰姬瑪哈陵(Taj Mahal)
11 月 18 日(一)	課程 19.如何對新的植物種類進行超低溫冷凍保存 實習 14.保存庫種原的病毒檢測 研習後評量測試 參觀植物遺傳資源博物館

11 月 19 日(二)	課程 20.體外基因庫和超低溫保存庫一全球現狀與展望 訓練課程總結報告、頒發結業證書、結業典禮 前往機場搭機
11 月 20 日(三)	清晨抵達香港國際機場，轉機返國至桃園國際機場

三、 研習內容紀要

(1) 印度國家植物遺傳資源局(NBPGR)植物遺傳資源保存

印度國家植物遺傳資源局的沿革起自 1935 年，但直至 1977 年才正式更名為「國家植物遺傳資源局」（NBPGR），及組織任務包括進行國外引進及國內種原收集保護，並辦理及研發種原庫內的長期保存措施及方法，對改善印度作物品種及農業多樣化發展發揮重要作用。它也是全球重要作物保存中心之一，目前規模僅次於美國國家植物種原系統(National Plant Germplasm System)，共有 441,253 樣本保存量(accessions)。其配置有國家種子庫、國家植物組織培養體外保存庫、超低溫保存庫、田間種原圃(圖 1~圖 8)及不同氣候區的 10 個工作站等棲地外保存設施。其各保存庫的作物保存情形歸納如下表。

表一、NBPGR 國家種子庫(seed genebank)的保存情形 (Apr. 31. 2019)

作物類別(Crop croup)	樣本保存量 (No. accessions)
穀物	164402
小米	59270
飼料作物	7095
假穀類(Pseudo cereals)	7618
豆類	66763
油籽 (oilseeds)	59200
纖維	15715
蔬菜	26383
水果和堅果	276
藥用、芳香、麻醉植物	8068
觀賞植物	659
香料和調味品	3152
農林業	1646
安全保存備份	10235
試驗材料	10771
合計	441253

表二、NBPGR 植物組織培養體外保存庫 (In vitro genebank)保存情形。(載至 Apr. 31. 2019)。

作物類別(Crop croup)	種質數量
熱帶果樹	430
溫帶果樹	355
塊莖作物	518
球莖作物	171
藥用、芳香植物	173
香料和工業作物	277
總計	1,874

表三、NBPGR 超低溫保存庫 (Cryobank) 的保存情形 (Sept.31.2019)

作物類別(Crop croup)	種質數量
異儲型(recalcitrant)和中間型(intermediate)	6837
正儲型(Orthodox)	3909
休眠芽	389
花粉	578
DNA	2114
總計	13820

表四、NBPGR 田間種原圃(Field genebank)的保存情形

作物類別(Crop croup)	種質數量
芒果	2727
香蕉	2451
柑橘類	928
番石榴	343
菠蘿蜜	300
葡萄	600
番木瓜	120
人心果	166
荔枝	85

(2) 超低溫冷凍保存技術(Cryopreservation)

超低溫冷凍保技術是指利用超低溫度(通常是在液態氮中(-196C))對植物遺傳資源進行保存，其具有所需空間小、勞力、經費及能源消耗少、能長期保存、不影響遺傳穩定性及不影響植物組織活性等優點。最早是由 Sakai 於 1960 年發表

對銀樺進行超低溫冷凍保存，目前已成功地應用在超過 160 個品種的冷凍保存上。其基本原理是在進行超低溫冷凍保存前，先降低細胞內的水分含量，避免冰晶形成對細胞造成傷害，同時提升植物組織的耐凍性，以減少組織細胞的死亡。進行保存的材料可以選用種子、體胚、癒傷組織、或莖頂等。早期傳統以冷凍誘導脫水，但因需使用特殊冷凍設備逐步降溫，花費昂貴，新技術則以誘導細胞內呈玻璃質化為基礎，目前常使用的超低溫冷凍保存方法包括有玻璃質化法(vitrification)、藻膠包埋乾燥法(Encapsulation)及藻膠包埋玻璃質化法(Encapsulation vitrification)、藻膠包埋脫水法(Encapsulation- dehydration)、液滴玻璃質化法 (droplet vitrification)、脫水冷凍盤法(D-cryoplate)及玻璃質化冷凍盤法(V- cryoplate)。表五為國際重要種原保存組織針對不同作物選用之冷凍保存方法，以休眠芽冷凍保存法及液滴玻璃質化法最常被使用。

表五、國際重要種原保存組織的保存作物類別及冷凍保存選用方法

Institute	Country	Crop	Cryopreservation Method
Bioversity International, Leuven	Belgium	Banana	• Droplet vitrification
Crop Research Institute, Prague	Czech Republic	Potato, garlic, hops	• Droplet vitrification
International Center for Tropical Agriculture (CIAT), Cali	Colombia	cassava	• Droplet vitrification • Encapsulation/dehydration
International Institute of Tropical Agriculture (IITA), Ibadan	Nigeria	Yam, banana, cassava	• Droplet vitrification
International Potato Center (CIP), Lima	Peru	Potato	• Straw vitrification • Droplet vitrification
Julius Kühn-Institut (JKI), Institut für Züchtungsforschung an Obst, Dresden	Germany	Strawberry/ Fruit trees	• Vitrification • Dormant bud freezing
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department, Gatersleben	Germany	Potato, garlic, mint	• Droplet freezing • Droplet vitrification
National Agrobiodiversity Center (NAAS), RDA, Suwon	South Korea	Garlic	• Droplet vitrification
Tissue Culture and Cryopreservation Unit, NBPGR, Delhi	India	Banana, chives, medicinal plants, berries, fruit trees.	• Vitrification • Droplet vitrification • Slow freezing • Dormant bud freezing
USDA-ARS, Fort Collins and Corvallis	USA	Citrus species, grape, garlic, mint, fruit trees.	• Vitrification • Droplet vitrification • Slow freezing • Dormant bud freezing

(3) 超低溫冷凍保存技術的使用現況

針對國際上 26 個具有冷凍保存設施的種原保存機構進行調查，合計約有 30 種作物 9650 個樣本數(accessions)利用冷凍保存，約占所有樣本保存量的 16%，以馬鈴薯、樹薯、桑科作物及香蕉的冷凍保存樣本最多，相較之下冷凍保存的作物種類仍是比較狹隘，這些冷凍保存樣本有 66%同時以田間種原圃及 46%以體外組織培養方法進行保存。在運作上缺乏預算、有經驗的操作人員及適用方法

為這些機構在進行冷凍保存時的主要的困難點。預估截至 2022 年冷凍保存的樣品量會增加至 15526 樣本數，同時約有 100,000 個不同的樣本預計需要以冷凍技術進行長期冷凍保存。超低溫冷凍保存技術是目前對異儲型種子及無性繁殖作物進行長期遺傳資源保存最有效的方案，但在過去的發展中，以開發溫帶作物的保存方法較為成熟，對熱帶和亞熱帶物種的保存方法開發大幅落後。有鑑於熱帶作物有許多異儲型種子及無性繁殖作物，因此其方法開發尤顯重要

四、 研習照片

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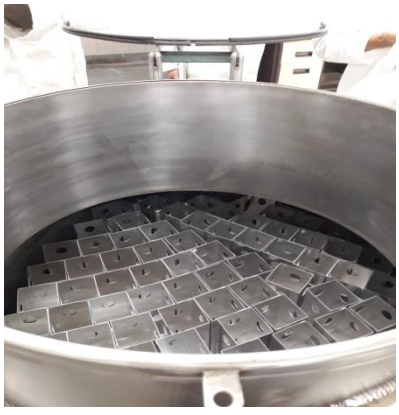
<tr><td>圖 3. NBPGR 組織培養體外保存種原庫</td><td>圖 4. NBPGR 組織培養體外保存種原庫</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>圖 5. NBPGR 超低溫冷凍保存庫</td><td>圖 6. NBPGR 的超低溫冷凍保存庫內部</td></tr>
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圖 7.NBPGR 香蕉田間種原圃	圖 8.NBPGR 波羅蜜田間種原圃
	
圖 9.開幕式、貴賓致詞與學員介紹	圖 10. ICAR 貴賓與 NBPGR 人員與學員合影
	
圖 11. APCoAB 的 Dr RK Tyagi 進行授課	圖 12.學員上課與 Dr Tyagi 互動情形
	
圖 13.印度國家研究員 Dr. Sunil Archak 進行 PGR 資訊管理演講	圖 14. ICAR 國家植物遺傳資源局局長 Dr. Kuldeep Singhru 演講

	
圖 15.本次訓練的主辦人 Dr Anuradha Agrawal 進行授課	圖 16.Dr.Sharma 進行玻璃質化冷凍保存解說
	
圖 17. 學員進行實驗操作	圖 18. Dr Agrawal 示範香蕉莖頂分生組織取樣
	
圖 19. 超低溫冷凍保存庫解說	圖 20.超低溫冷凍保存庫取樣示範

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叁、心得及建議

一、植物遺傳資源超低溫冷凍保存庫是為未來世代需求做準備，一旦開始作業就無法停頓，因此在建立前需有審慎的成本及預算評估，並須確保液態氮能長期持續供應，另外需要有完整的種原紀錄(passport)，並配合良好的管理系統，才能達到持續利用的目的。目前國際間開始號召備份(back-up)計畫，希望能透過目前各國現有的種原庫資源進行彼此的備份保存。

二、種原保存的最終目的是再生與利用，因此任何保存方法的建立，必須基於確保植株可以被再生為前提。目前許多方法以活力(Viability)做為評估標準，可能會有高估的疑慮，植株的再生(re-growth)需以能長成完整植株作為謹慎評估。

三、超低溫冷凍保存法雖為營養繁殖作物及不耐儲存型種子的良好方案，但現階段仍以溫帶作物有較多的成功案例，熱帶或亞熱帶作物因本質較不耐低溫和乾燥，目前僅香蕉、柑橘、桑葚、荔枝、波蘿蜜等有成功的例子，因此許多熱帶作物的冷凍保存方法仍待進一步開發。

四、芒果的種原保存是目前的棘手案例，不管是利用組織培養的體外保存或是超低溫冷凍保存，一直仍有困難無法克服，現階段以花粉做為保存方案以作為育種的輔助工具，未來仍有待開發新的方法以突破困境。

五、在體外保存和超低溫冷凍保存種質的遺傳穩定性研究方面，目前雖有型態、染色體穩定性、代謝物類型及 DNA 序列變異的分析方法，但在 epigenetic change 的偵測上，尚缺乏快速簡易的分析方式，是未來相關領域可開發的方向。

六、本次訓練包含有授課與實作課程，於研習前、後分別進行了評量測試用以評估學習成效，結果顯示學員的學習成效於訓練後平均提升了 27%。對於學員而言，評量雖是一種壓力，但可充分讓自己了解吸收了多少，或有哪些觀念尚未釐清，能幫助正向學習。未來在辦理相關訓練時建議也可採用此種方式，幫助了解學習成效及達成正向學習。

七、本次訓練期間與亞、非等各國學員積極互動，建立友誼，並就運用分子技術於種原管理及熱帶林木、熱帶作物(如甘藷、芒果及甘蔗等)種原保存方法的開發與菲律賓、印度等新南向國家熱烈討論，期許未來能有更進一步的技術交流。

肆、附錄

Collecting Plant Genetic Resource for Conservation, Research and Use

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Collecting Plant Genetic Resources

Plant genetic resources as genetic material of actual and potential value, consist inter and intra-specific diversity including landraces and primitive cultivars of crops and their wild relatives. Indian gene centre is one of the 12 mega-biodiversity centres of crop plants with four hot-spot areas (Himalayas, Indo-Myanmar region, Western Ghats and Andaman & Nicobar). It has over 17,500 species of higher plants and 500 species of cultivated plants playing a vital role in food, fodder, fiber, medicine, aesthetic, commercial and industrial uses. Plant genetic diversity is essential for success of crop improvement programme. This wealth is depleting at an alarming rate due to natural and man-made activities. Therefore, ICAR- NBPGR is entrusted to explore and collect the germplasm variability in different agri-horticultural crops, their wild relatives and related species for their conservation and utilization in crop improvement.

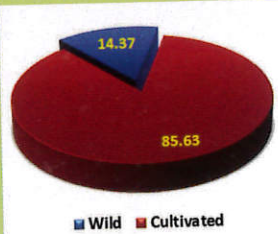


Fig. 1 Precent addition in culitivated vs wild accessions (1946-2017)

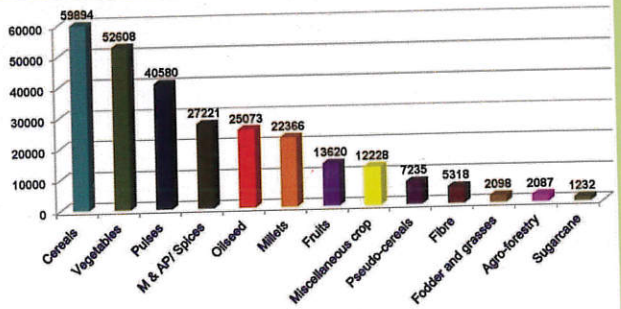


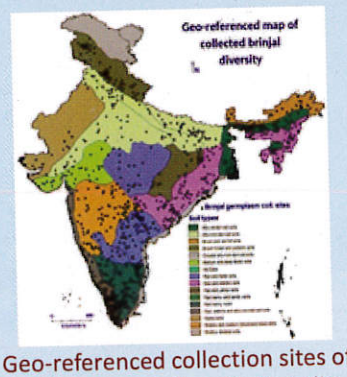
Fig. 2 Germplasm Collected in Different Crop-groups (1946-2017)

Salient Achievements

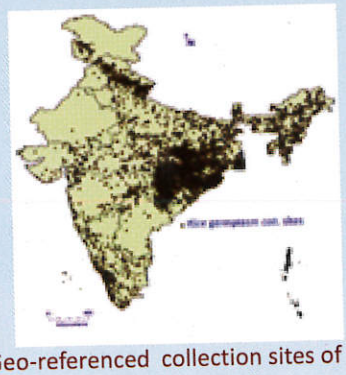
- 2,71,600 (0.27 million) germplasm accessions (85.63% cultivated, 14.37% wild, Fig. 1) representing over 2,000 crops /species have been assembled through ~2695 exploration trips undertaken in collaborative mode involving crop-based ICAR institutes, State Agricultural Universities (SAUs), Krishi-Vigyan Kendras and other organizations.
- Crop-group wise (14 groups) accessions collected are depicted in Fig.2.
- Unique germplasm collections added in rice: rare and deep-water landraces- *Batu, Indi, Mia, Dol, Jul, Dhepa, Tulsi, Dubari, Ikarasali* and *Dhusuri* from Assam; cold tolerant landraces- *Lahi, K.Botha, Sikota Lahi* and *Dal Boradhan* from Arunachal Pradesh, good-to-taste landrace '*Signal*' from Tripura; salt tolerant landraces- *Getu, Nonabokra, Talmugur, Darsal, Marisal* and *Dudheswar* from Sundarbans (West Bengal); early maturing drought-tolerant landrace '*Sathee*' (60 days) from Jharkhand and Chhattisgarh.
- A total of 718 wild relatives of agri-horticultural crops have been short listed from India, of which ~100 species are prioritized for germplasm collection. In all 250 species (~10,000 accessions) collected include ~98 closely related wild relatives of different agri-horticultural crops.
- Diversity distribution and collection maps were prepared in nine crops viz. rice, wheat, maize, *Brassica* spp., pigeonpea, sesame, sorghum, pearlmillet and tomato using modern tools of GIS and GPS (Fig.3). Gaps in germplasm collections in these crops have been identified to plan and undertake future explorations.

Variability in panicle size of foxtail millet

Native diversity in brinjal collected from different parts of country



Geo-referenced collection sites of *Solanum* spp. diversity in India



Geo-referenced collection sites of rice diversity in India

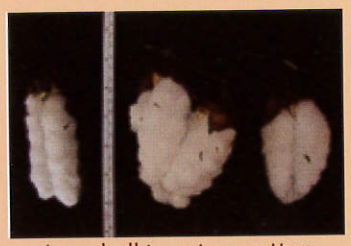
Cajanus cajanifolius & *C. scarabaeoides* -close relatives of pigeon pea

Novel/ Trait-specific Germplasm Collected (in last five years)

- Early maturing drought-tolerant rice landrace '*Sathee*' (60 days) from Jharkhand
- Popcorn with cylindrical, thin and conical-shaped cobs and high yielding landrace (*Akimu-Khulati*, 16 rows, 48-50 seeds/row) from Nagaland
- Orange-fleshed (carotene rich) cucumber from Manipur and Mizoram
- Brown-netted cold tolerant cucumber from Meghalaya
- Scented spongegourd from Tripura
- Linear-elongate ashgourd with small seeds from Manipur
- High yielding teaselgourd (*Bhat karela*) from Tripura
- High pungent bird-eye chili from Mizoram
- Very tasty Frenchbean '*Keshwan rajma*' from remote area of Kistwar (Jammu & Kashmir)
- Tree cotton (*Gossypium arboreum*) with long boll (11cm) from Mizoram and light brown-linted (naturally coloured) from Tripura
- Cut-leaf mustard (*Brassica juncea* subsp. *integrifolia*) from Arunachal Pradesh
- Prolific bearing wild *Moringa oleifera* from foothills of Punjab
- Wild relatives of wheat and barley (*Elymus/ Leymus/Hordeum*) from Leh and Kargil (J&K) and pulses (*Vigna angularis* var. *nipponensis*) from Arunachal Pradesh and Mizoram (*Cajanus cajanifolius*) from Bastar and Odisha.

Cobs of Sikkim primitive maize

Popcorn type cylindrical cobs from Nagaland



Long boll type tree cotton from Mizoram

Orange-fleshed carotene rich cucumber from Manipur

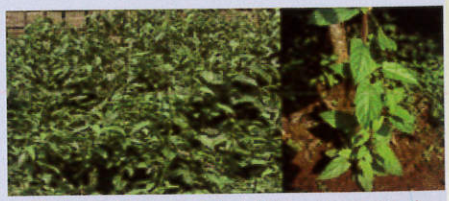
Variability in panicles of *Elymus nutans* - wild relative of wheat from Leh district

New Species/Uses and New Records of Distribution

- Described as a new species, *Herpetospermum operculatum,* used as leafy vegetable in Nagaland (distributed in India, Myanmar and China).
- First time collected, rare and endangered species includes *Citrus ichangensis* (Nagaland), *Trichosanthes truncata* (Arunachal Pradesh), *T. himalensis* (Sikkim), *T. cordata* (Manipur and Assam), *T. nervifolia*, *Gymnopetalum wightii* (south Western Ghats) and a taxon new to India *Momordica subangulata* subsp. *subangulata* (Nagaland).
- Occurrence of crop wild relatives viz. *Luffa echinata* in Haryana; *Hystrix duthie* in Jammu & Kashmir and *Cucumis javanicus* in Meghalaya, *Solanum diphyllum* in Delhi and Andhra Pradesh; *Allium fasciculatum* in Arunachal Pradesh was first time recorded.
- ***New record of cultivation***: *Plukenetia corniculata* 'Meetha-patta' - used as leafy vegetable in Assam and Nagaland, *Cucumis metulifer* and *Canna indica* as salad and tuber crop, respectively in Darjeeling and Sikkim, *Abelmoschus manihot* ssp. *tetraphyllus* 'Sukhlai'as clearing agent in jaggery industry in western U.P. and terai region of Uttarakhand.

Herpetospermum operculatum- a new species from NEH region

Indian wild orange (*Citrus indica*) locally called "*Bango Shinkin*" with small fruit from Arunachal Pradesh



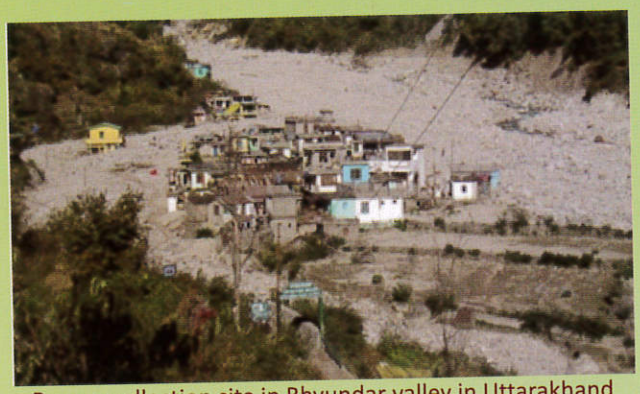
Plukenetia corniculata' Meetha patta'- a new crop under cultivation in Assam & Nagaland

Special Exploration Missions Executed in India

Seventeen special missions were executed in diversity rich areas and where diversity was under imminent threat. In these missions, nearly 8,000 accessions of crops landraces and species of potential value were gathered. This diversity, else, would have been lost due to the threats.

(a) Rescue missions

- Cyclone-hit areas (Odisha, 1999-2000)
- Tehri Dam catchment areas (Uttarakhand, 2000)
- Earthquake-hit areas (Gujarat, 2001)
- Sardar Sarovar and adjoining catchment areas (Gujarat, Madhya Pradesh and Maharashtra, 2001-2003)
- Flood-affected areas (Odisha, 2008)
- Natural calamity affected areas (Uttarakhand, 2013)



Rescue collection site in Bhyundar valley in Uttarakhand

(b) Diversity-rich areas explored

- Majuli River Island (Assam, 2000-2001)
- Lohit district (Arunachal Pradesh, 2000)
- Brahmaputra River Islands and Char areas (Assam, 2003)
- Valley of Flowers (Uttarakhand, 2000)
- Kalakkad-Mundanthurai Tiger Reserve (KMTR, Tamil Nadu, 2000-2001)
- Eastern Ghats (Tamil Nadu and Andhra Pradesh, 2001-2003)
- Andaman & Nicobar Islands (2003)
- North Eastern Region (Special drive, 2011-16)

Tsunami affected rescue collection site in Odisha

Special Exploration Drive to North Eastern India (2011-16)

- A total of 3,103 germplasm samples were collected in forty five explorations carried in 80 districts (out of 105) of all the eight states of NE Region in collaboration with 16 crop-based institutes of ICAR/ SAUs, 58 KVKs and 130 officials from State Agricultural Departments/ Agricultural Colleges.
- Diversity was collected in 190 taxa of cereals and millets (rice, maize, foxtail), pulses (cowpea, ricebean, soybean), pseudocereals/underutilized (buckwheat, job's-tear, perilla), and vegetables include, leafy mustard, chilli, brinjal, garlic and cucurbits (ash gourd, bottle gourd, cucumber, pumpkin, teasle gourd and *Luffa* besides, species diversity in *Momordica, Solanum, Trichosanthes*). Local races of *Gossypium arboreum*, known for various traits were collected from Mizoram and Manipur.
- Diversity in *Citrus* (18 spp.), including its threatened wild species, was collected from Arunachal Pradesh, Assam, Tripura and Nagaland.
- Indigenous knowledge on minor economic species for their edible and medicinal value was documented from Barak Valley (Assam), Manipur, Mizoram and Nagaland.

Variability in grain & kernel of rice from Tripura

National Herbarium of Cultivated Plants (NHCP)

- A total of 22,566 herbarium specimens and ~6,000 digitized images are available in National Herbarium of Cultivated Plants (NHCP) for study and referral use by students and researchers. Providing technical inputs on identification, authentication and taxonomic know-how on taxa of PGR relevance using NHCP facility.

Digitized images of herbarium specimens in NHCP

Taxonomic and Systematic Study

- Taxonomic and systematic studies conducted in *Allium*, *Trichosanthes*, *Luffa* and leafy amaranth to resolve issues on species identity, taxonomic delineation and to workout relatedness among species using morphological and molecular tools.

Biosystematic studies in *Trichosanthes* through grow out test

Local Knowledge/Information Documented

- Flowers of *Crotalaria tetragona (Tum-thang)* are used by tribals of Mizoram to garnish local and non-vegetarian dishes.
- Leaves of *Bidens pilosa* are used in preparation of local /Ladakhi tea (*Jya*) in Ladakh region. The plant is cultivated in Nubra Valley, Ladakh.
- Seed/Kernel of *Hodgsonia heteroclita* (*Kathai*) containing >70% oil, is used as food and medicine by tribal communities of NEH Region.
- Documented an ITK on *Jumin*: a traditional beverage prepared using *Aaho* rice landrace and foxtail millet by *Nocte* tribe of Arunachal Pradesh.
- Documented ITK on fibre extraction method of sunnhemp in Bundelkhand region of U.P.
- Besides, nearly 200 ITKs related to the Plant Genetic Resources were recorded during explorations.

Flowers of *Crotalaria tetragona*' Tum-thang' in local market of Mizoram

Sale of minor fruit in local market by tribal women in Gujarat

Recording indigenous knowledge from tribal womenfolk in NEH region

Explorations Carried out in other Countries

Explorations were carried out in 18 countries (prior to CBD) under bilateral collaborations with FAO, US-AID, and IPGRI (now Bioversity International) for enriching diversity in major cereals, millets, legumes, vegetables (cucurbits, okra, brinjal), tuber corps and crop wild relatives.

Impact of Germplasm Collection Activity

- Exploration and collection has conserved the diversity of a large number (~1218) of crops/species, which otherwise would have lost due to genetic erosion, habitat destruction and climate change. It has provided the basic material for breeding and improvement in crops and to meet food and nutritional security.
- Most of the varieties in fruits, vegetables, millets, pulses, medicinal and aromatic plants are developed through selection of collected/ introduced germplasm, and significantly contributing in the food security and national economy.
- Collaboration with stakeholders (crop based ICAR-Institutes, SAUs, KVKs) have mutually benefited by sharing germplasm, enriching knowledge on landraces/ local material and updating diversity status of species/crops in areas/habitats.
- Explorations in unexplored/difficult and remote areas like Mon, Kiphire, Anini, Anjaw, Tirap, Changlang, Gurej valley, Janskar, Ladakh, Great Nicobar, Gadhchiroli, Bastar, Sundarban, etc. have helped in collection of rare, underutilized germplasm, potentially useful material (*Allium* spp., *Herpetospermum operculatum, Hodgsonia heteroclita, Plukenetia corniculata, Cucumis metulifer, Vigna angularis* var. *nipponensis, Cajanus cajanifolius* and many more).

Awareness meeting of farmers under TSP programme

- Biodiversity/farmers fairs and awareness programmes have created awareness on conservation of agro-biodiversity in tribal dominated and remote areas covering ~25,000 farmers of 4,875 villages. Live demonstrations and publications have further enhanced the awareness on importance of crops genetic resources and need for conservation. During explorations, the farmers were explained about importance of germplasm and consequences of its loss.
- Over 1,200 scientists, researchers and students were benefited from taxonomic and systematic knowledge through visit to National Herbarium of Cultivated Plants which in turn extended inputs in various research programmes.

Thrust Areas

- Gap analysis, geo-referencing and mapping of collected diversity.
- Germplasm collection of landraces, crop wild relatives, minor fruits/wild economic species including trait-specific germplasm (biotic, abiotic stresses and quality).
- Exploration in unexplored/inaccessible/tribal/under-explored areas and diversity rich pockets.
- Taxonomic and bio-systematic studies in native taxa.
- Documentation of IKs, on-farm diversity studies and awareness generation on agro-biodiversity.
- Build-up of herbarium specimens and digitization of CWR, diverse germplasm of PGR importance .
- Human resource development (teaching/ training)

Copies available from: Head, Division of Plant Exploration and Germplasm Collection

Compiled by

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For more information visit! http://www.nbpgr.ernet.in

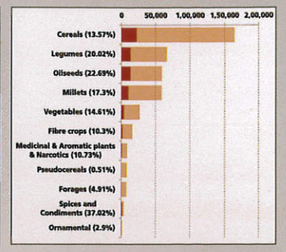
Maintenance of base collection

- The base collection is maintained at -18 °C in 12 long-term storage modules at ICAR-NBPGR, New Delhi.
- Germplasm accessions are routinely monitored for loss of viability and fall in seed quantity at pre-determined intervals.
- Those accessions where viability percentage has dropped below 85% of initial viability or are of less seed quantity are regenerated following appropriate standards in association with the National Active Germplasm Sites (NAGS) under the INPGRN.

Indian National Plant Genetic Resources Network

Monitoring

Regeneration of wild wheat under extended photo period

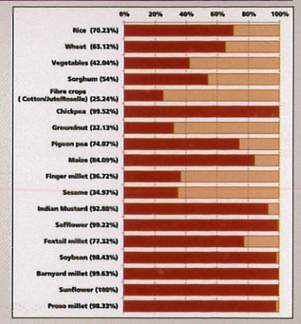


Regeneration status in NGB (as on June 2018)

Value addition for enhanced utilization

Characterization is key in determining the value of a conserved collection and is a prerequisite for trait discovery. A significant number of accessions in NGB have been characterized largely under the Consortium Research Platform on Agrobiodiversity and other projects such as NICRA and NRRD in collaboration with the INPGRS.

Characterization of wheat germplasm



Characterization status of major crops in NGB (as on June 2018)

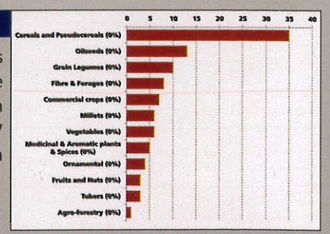
Large scale screening of germplasm in NGB have been undertaken for trait discovery in wheat, sesame and millets.

Crop	Number of accessions	Trait
Wheat	21,258	Tolerance to terminal heat stress
Wheat	20,660	Identified 498 accessions resistant to multiple rusts; Identified 868 accessions resistant to spot blotch; Identified 56 accessions resistant to all three rusts and spot blotch; Identified 125 combinations of Lr, Sr and Yr genes and a spot blotch QTL.
Sesame	1,925	Screening for identifying new potential crops for Punjab.
Millets	5,009	

Mega screening trials for trait discovery

Plant Germplasm Registration System

The activity of registration of unique germplasm was started in 1996 with the objective of giving credit to the developers and bringing the trait specific germplasm in public domain. Since the initiation of this mechanism, 37 meetings have been held and a total of 1,377 germplasm belonging to 215 species have been registered.



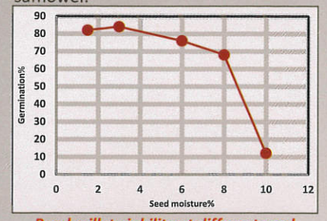
Status of Registered Germplasm (as on 2018)

Supportive research/ Other activities

- Dormancy breaking protocols have been standardized in 75 species to aid their conservation in NGB.

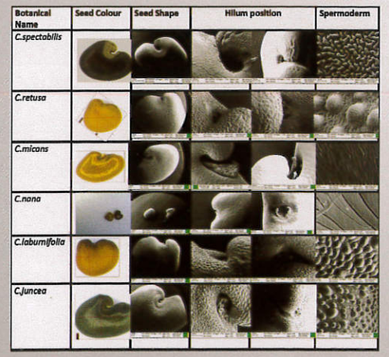
Crop Group	No. of species
Medicinal and Aromatic Plants	41
Fibre & Forage	25
Vegetable	9
Pulses (*Vigna* spp.)	10

- Strategies for low-energy conservation of ultra desiccated seeds under ambient conditions were evaluated and standardized in chickpea, sesame, niger, castor and safflower.



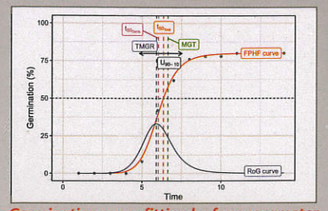
Pearl millet viability at different seed moistures after 25 years of storage at 5°C

- Conservation initiated for populations and mutants.
- Identification keys for wild species of *Swertia* and *Crotolaria* using seed characters.



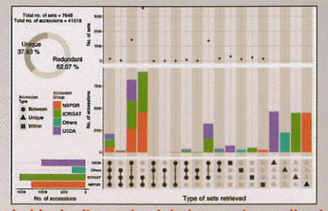
Identification key developed for Crotolaria

- Software packages 'viabilitymetrics' and 'geriminationmetrics' were developed to aid computation of germination indices, viability statistics and curve fitting of cumulative germination and seed survival curves.



Germination curve fitting by four parameter hill function

- A software package 'PGRdup' was developed for the identification of probable duplicate accessions from plant genetic resources passport databases by fuzzy, phonetic and semantic matching of extracted keywords.



Probable duplicates in global groundnut collection

- 25 pigeon pea 100 rice and 100 sorghum accessions have been deposited in the Global Seed Vault in Svalbard, Norway under permafrost conditions as safety duplicates.

- NGB designated as the official repository for NBA material.
- Efforts have also been initiated to develop a similar facility in India in collaboration with DRDO and a prototype facility has been established at Chang-la in Ladakh.

Prototype of National Permafrost Facility at Chang-la

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National Seed Genebank

Conserving diversity For today and posterity

Repository of > 4 lakh accessions belonging to 1,900 species

In the service of nation since 1986

Second largest genebank in the world

ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110012
www.nbpgr.ernet.in

Introduction

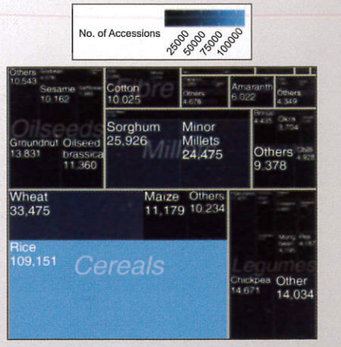
The National Genebank (NGB) has been established in 1986 at the National Bureau of Plant Genetic Resources, New Delhi, for ex situ conservation of germplasm collections in the form of seeds, vegetative propagules, tissue/cell cultures, embryos, gametes, etc. Amongst these, seeds are the easiest and most convenient form for long-term conservation/ distribution and hence the seed genebank forms the major component of NGB and the cornerstone of the Indian National Plant Genetic Resources Network (INPGRN).

Seed Genebank at NGB

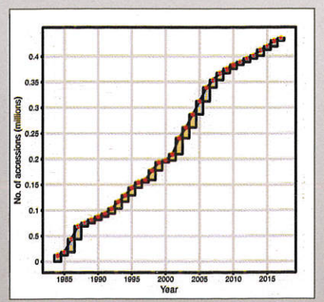
Germplasm Assembly

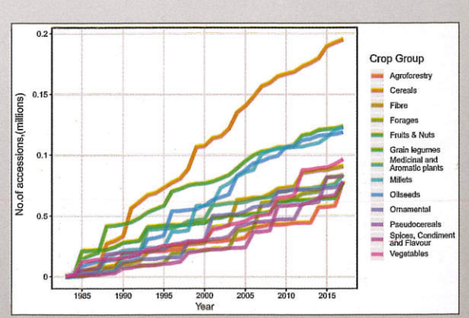
Over the last three decades NGB's seed genebank has grown into a colossal seed vault and has been catering to the crop improvement programmes of almost all agri-horticultural crops. Valuable germplasm of various crop species has been collected, acquired and assembled in NGB from all over India and the world through an active collaboration with the partners in the national agricultural research system (NARS). As a result of all these efforts, the base collections in the NGB holds 4,38,084 accessions belonging to 1,900 species as on June 2018, making it the second largest genebank in the world, next only to National Plant Germplasm System, USA (USDA Genebank).

Crop/Crop Group	No. of Species	No. of Accessions
Cereals	117	164039
Legumes	97	65911
Oilseeds	76	58719
Millets	24	58557
Vegetables	208	26168
Fibre crops	68	15665
Medicinal & Aromatic plants & Narcotics	665	7978
Pseudocereals	54	7405
Forages	191	6960
Spices and Condiments	26	3101
Agroforestry	189	1644
Ornamental	120	656
Fruits & Nuts	65	275
Trial Material (Wheat, Barley)	-	10771
Duplicate safety Samples (Lentil, Pigeonpea)	-	10235
Total	1900	438084

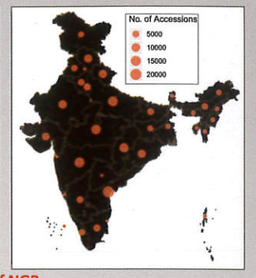


Status of germplasm conserved in seed genebank of NGB (as on June 2018)



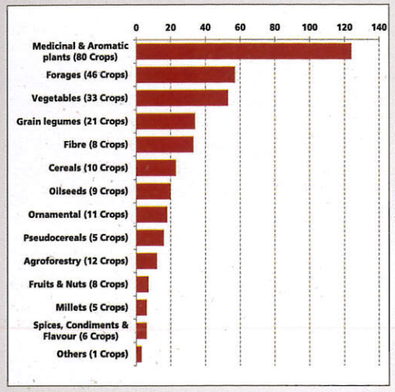


Growth of collections in seed genebank



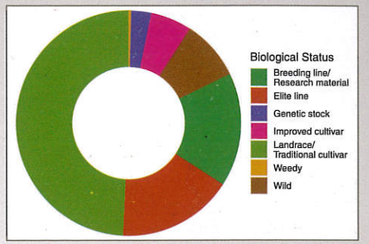
Origin of accessions held in seed genebank of NGB

The NGB collection has more than 75,000 landraces/traditional cultivars in multiple crops, which are not currently in cultivation and are the original genetic back up for every potential trait. The 25,000 elite lines, 9,300 improved cultivars and 4,500 genetic stocks are also unique material that can be readily utilized by breeders in respective crop improvement programmes.



New crop species added in seed genebank of NGB (2008-2018)

Several core and mini-core collections for facilitating trait discovery in different crops, have been conserved in NGB, both developed in house in ICAR-NBPGR as well as from other institutes



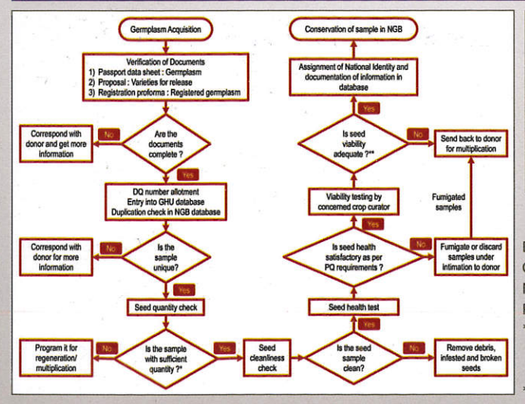
Accessions with known biological status in seed genebank of NGB

Further, over the last ten years, over 400 species across different crop groups have been added to the NGB collections.

Crop	Core/ Minicore/ Reference sets	Source/ Developing Institute
Wheat	Core (2226), Mini core (228), Reference set for terminal heat tolerance (3019)	ICAR-NBPGR
Rice	Core (1548), Mini core (314)	ICAR-NBPGR
	Core of North Eastern collection (700)	ICAR-NBPGR
Brinjal	Core (181)	ICAR-NBPGR
Safflower	Core (620), Mini core (150)	ICAR-IIOR
Groundnut	Mini core (167)	ICAR- NRCG
	Mini core (184)	ICRISAT
Sesame	Core (329)	ICAR-NBPGR
Sunflower	Core (99)	USDA
Chickpea	Core (1103)	ICAR-NBPGR/
	Mini core (211)	MPKV, Rahuri ICRISAT
Mung bean	Core (152)	ICAR-NBPGR
Pigeon pea	Mini core (146)	ICRISAT

Details of core/minicore/reference sets available at NGB

Accession Processing for Long-Term Conservation of Seeds

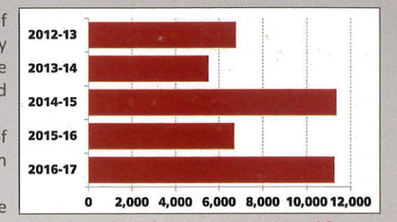


Flowchart of accession processing for long-term conservation of seeds in NGB

DQ : Domestic quarantine
GHU : Germplasm handling unit
NGB : National genebank
PQ : Plant quarantine
* Self pollinated (2000 seeds); Cross pollinated (4000 seeds); and wild (>500 seeds).
** > 85% (50-70% in wild species in vegetable/medicinal/rare/endangered/forage species)

Each seed sample is processed through a series of steps to ensure that it meets the quality requirements for long term storage as the base collection. This forms the core activity of the seed genebank.

- Arriving samples are checked for presence of adequate supporting documentation (passport data).
- Qualified samples are checked against the NGB database to detect duplication.
- Only samples of adequate seed quantity are processed further (>2000 seeds for self pollinated crops; >4000 seeds for cross pollinated crops and >500 seeds for wild species).
- Debris, inert material, damaged and infested/ infected seeds and seeds of other species (e.g., weeds) are removed to obtain clean and pure samples of seeds with potentially high physiological quality for storage.
- Clean seeds subjected to detailed seed health testing in Division of Plant Quarantine to ensure pest free conservation.



Number of accessions processed for conservation under LTS in the last 5 years (2012-2017)

Seed drying

- Seeds are dried in a two step process, first in walk-in seed drier and then in machine driers.
- Seed moisture is estimated by ISTA method.

Seed moisture estimation

- Adequately dried seeds are tested for seed viability to qualify the minimum requirements that ensure maintenance of seed viability under long term storage conditions of low temperature and seed moisture content. (> 85% in general and 50-70% for wild species in vegetable/ medicinal/ rare/ endangered/forage species).

Seed viability testing

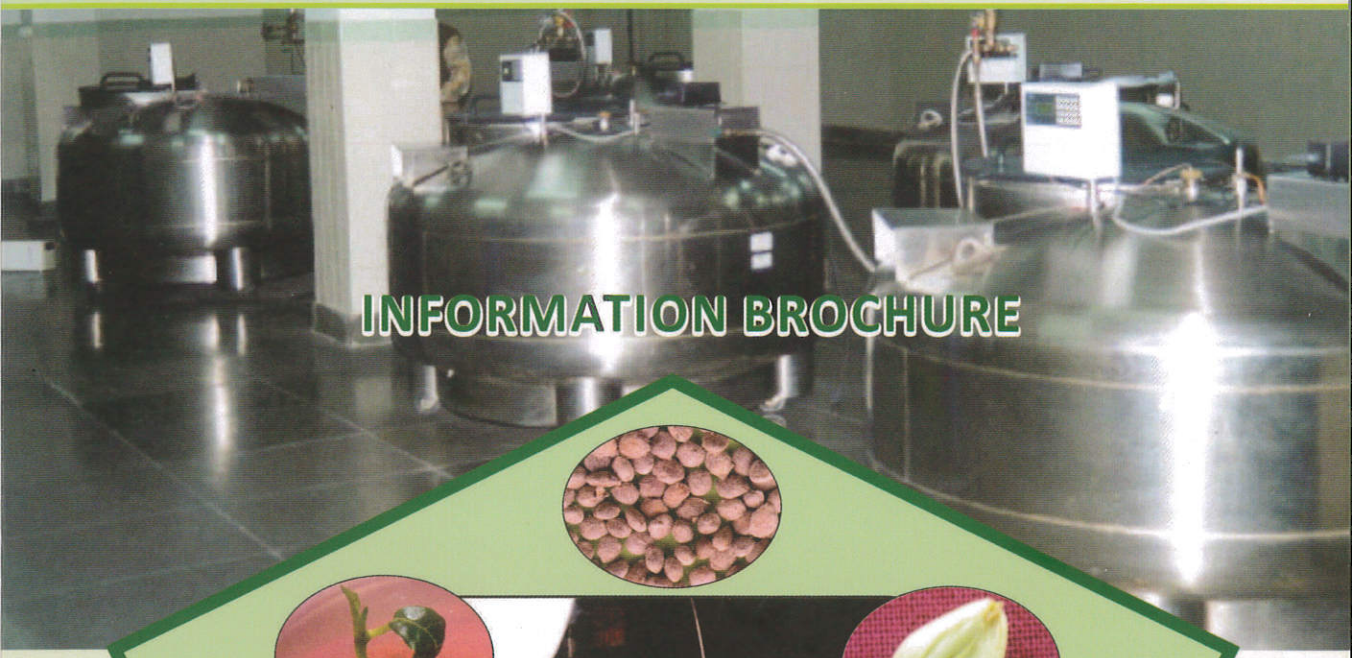
Seed packing

- After drying and viability testing, the seeds are packed in specially designed tri-layered aluminium foil packets (Outer polyester layer, 12 µm; Middle aluminium foil layer, 12 µm and Inner polyethene layer, 250 gauge) for long-term conservation in the base collection.
- Each accession is assigned a unique National Identity (IC or EC number) through Division of Plant Exploration and Germplasm Collection or Germplasm Exchange Unit in collaboration with Agricultural Knowledge Management Unit.
- The seed packets are labelled and stored at a particular location in the NGB.
- The genebank storage details such as storage location, seed quantity, moisture content and viability are updated and maintained in the Genebank database.

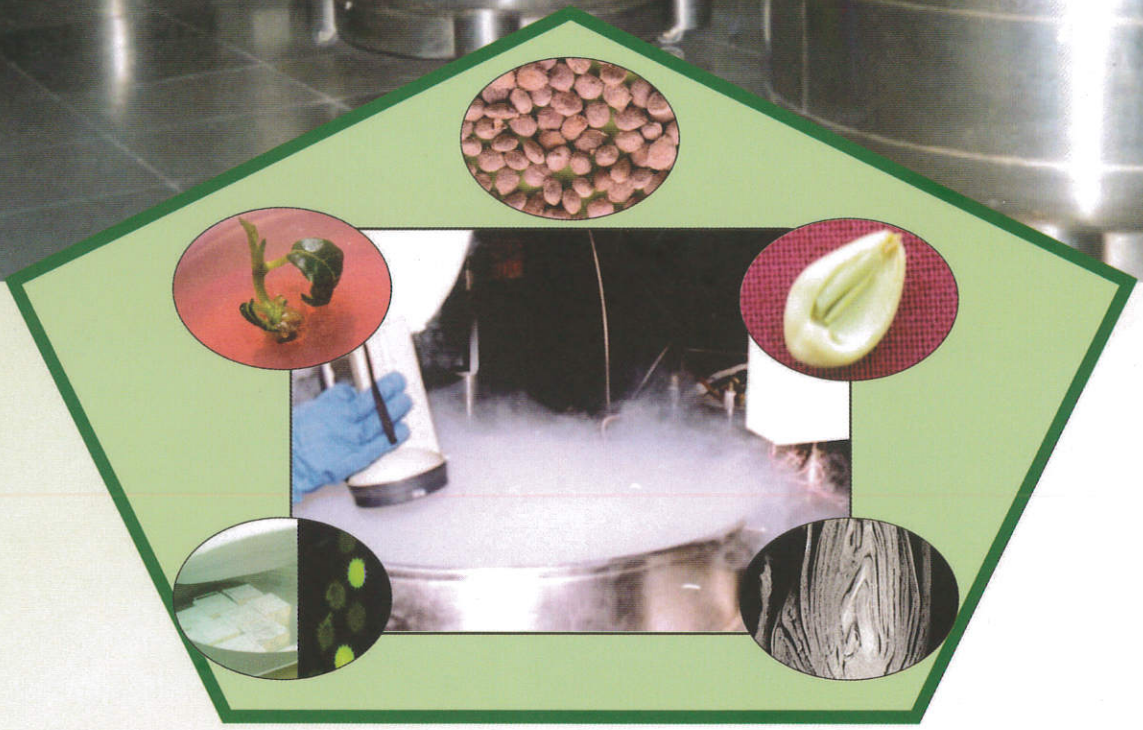
Packing

Documentation

National Cryogenebank



INFORMATION BROCHURE



Tissue Culture and Cryopreservation Unit

ICAR-National Bureau of Plant Genetic Resources

Pusa campus, New Delhi - 110012, India

Background

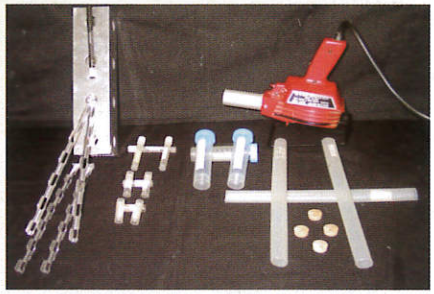
The word 'cryo' is a derivative of cryogenic which is defined as 'very low temperatures'. Stabilizing living cells at cryogenic temperatures, usually below -100°C is called **cryopreservation**. It is an applied aspect of cryobiology which is the study of life at low temperatures. Cryopreservation offers the opportunity of significantly reducing cell and tissue deterioration in storage by virtually halting metabolism and providing theoretically 'infinite' periods of storage to the materials. It is reported not to cause any change in viability, vigour and genetic make up of the conserved materials. Its wide adoption by leading genebanks of the world confirms its utility. It obviates the need to test stored materials frequently thus making storage cost effective.

National Cryogenebank

National Cryogenebank has been established at National Bureau of Plant Genetic Resources (NBPGR) since 1996 with the aim to conserve the national plant genetic wealth. However, the cryopreservation activities were already being carried out since 1986 to develop cryopreservation protocols. Presently this Cryobank has six extra large capacity cryotanks with state-of-the-art infrastructure capable of accommodating 30,000 to 40,000 samples of varied germplasm.

Cryopreservation technique

The storage of viable biological materials at ultra-low temperatures ranging from -150°C to -196°C has found applications in storing wide range of plant germplasm. The ultra-low temperatures are generated by the use of liquefied nitrogen (LN) in double walled vacuum insulated cryotanks. LN is inert, non-corrosive and non-inflammable and hence, preferred for use. This technology obviates the dependence on mechanical system and electricity. Samples are processed using specified protocols and then suspended in vapour phase of LN.



Inventory system for cryostorage

Cryopreservation: selective advantages

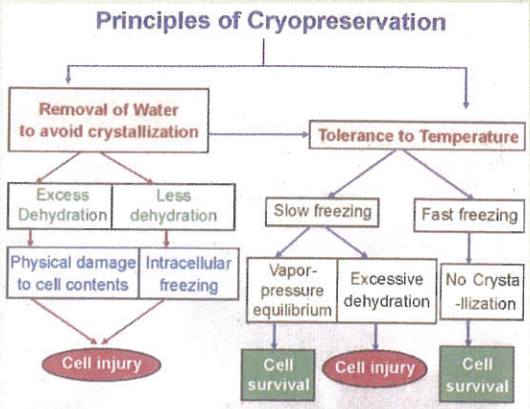
It is the only successful technique for long-term conservation of desiccation sensitive seeds, pollen and vegetative tissues. Cryopreservation is complementary to conventional seed storage at -20°C since germplasm of difficult-to-store species, unable to be stored in them, can be stored under cryopreservation. It is safe and economical method requiring minimal monitoring. At NBPGR Cryobank original viability values have been found retained as tested maximum upto 28 years of cryostorage.

Economic considerations

Considering the costs of acquisition, maintenance, processing and other general costs, cryopreservation has worked out to be the cheapest *ex situ* conservation method. The average cost per accession in cryopreservation is half of that in seed bank and field genebank and one fifth of that in *in vitro* conservation.

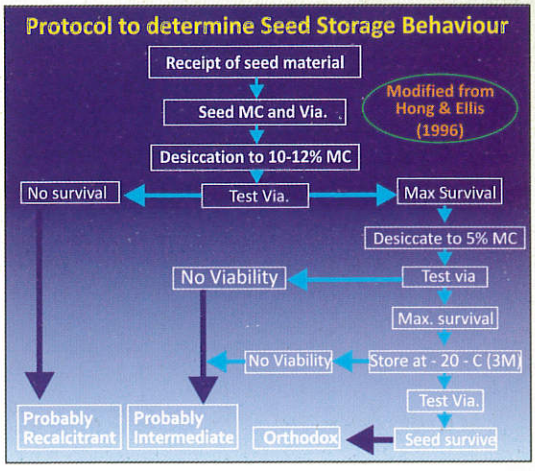
Germplasm priorities for cryobanking at NBPGR

- Species producing non-orthodox (intermediate and recalcitrant) seeds
- Vegetatively propagated species
- Medicinal and aromatic plants
- Threatened, rare and endangered plant spp.
- Released varieties
- Registered genetic stocks
- Wild and weedy relatives of crop plants



Practical considerations

For cryopreservation of plant germplasm explants like meristems, shoot tips, somatic and zygotic embryos, embryonic axes, whole seeds, dormant buds and pollen are the candidates. The explants are suitably desiccated to optimum moisture contents non-lethally before freezing. During cooling of samples a correct balance between dehydration injury and intracellular freezing is being maintained to ensure success in storage. Freezing rates are optimized for best responses. Rapid thawing / rewarming is done to avoid recrystallization and ice crystal growth.



Seed storage behaviour

Conservation in the form of seeds is considered to be the most reliable and preferred method. Understanding the seed storage behaviour of any species is a prerequisite for developing suitable cryopreservation methodology. Seed storage behaviour is analyzed by studying seed morphological, anatomical and physiological features especially desiccation and freezing sensitivity. Seeds, on the basis of their sensitivity to desiccation and freezing, can be classified into orthodox (desiccation tolerant), intermediate (desiccation and /or freezing sensitive), (desiccation and/or freezing sensitive), recalcitrant (desiccation and freezing sensitive). The seeds with small size and low moisture at the time of shedding e.g annual crops are typically orthodox and their longevity can be considerably enhanced by storage at -20°C temperature in traditional seed genebanks. Seeds with large size and high moisture content at the time of shedding are variably desiccation and freezing sensitive and hence categorized as non-orthodox i.e intermediate / recalcitrant. These seeds when desiccated between 12-30% moisture content show a considerable loss of viability and can be conserved only at ultra-low temperatures of LN. This seed storage behavior is mostly exhibited by perennial tropical, subtropical and several temperate tree species.

In case of intermediate seeds of neem, black pepper, almond, apricot, khirni and several *Citrus* species, embryos and /or embryonic axes have been successfully cryopreserved with high percentage recovery. While in truly recalcitrant species such as litchi, jackfruit, cocoa and mahua, cryopreservation of embryonic axes has been achieved with very low percentage recovery. There has been major emphasis on developing cryopreservation techniques for pollen of tropical spp. especially those producing recalcitrant seeds as an efficient alternative to seed conservation.

Recalcitrant seeds of *Madhuca indica*

Intermediate seeds of *Citrus* species

Orthodox seeds of *Malus* species

Vegetative tissues

Vegetatively and clonally propagated plant species can be conserved on medium-term basis using *in vitro* conservation methods. However, for long term conservation, cryopreservation is the only alternative. Shoot apices, meristems, somatic embryos, dormant buds, etc, are desiccation and freezing sensitive to varying degrees. In temperate species during autumn cold hardening process is observed which enables plants to survive extreme low temperatures of winter. Winter dormant buds of such species are amenable to cryostorage. Several techniques are now available for long-term conservation of these explants using cryopreservation.

New Cryopreservation Techniques

Several vitrification-based techniques like pre-growth-dehydration, desiccation-freezing, vitrification, encapsulation - dehydration, encapsulation-vitrification and V-cryoplate method are being used for conserving the desiccation sensitive tissues. Significant progress has been made in developing cryopreservation protocols for several tropical and temperate fruit sp. and nuts by these techniques.

Collaborative research activities

In our efforts to expand the cryopreservation activities in plant genetic resources, collaborative research work has been undertaken with ICAR and with other departments. The institutes having field genebanks of priority species, especially those dealing in horticultural and forestry species, seek NBPGR's support for developing efficient protocols for cryostorage to enable their long- term conservation. In several cases basic training on aspects of cryostorage are extended to scientists of these institutes at NBPGR to help them in initiating research activities for development of suitable

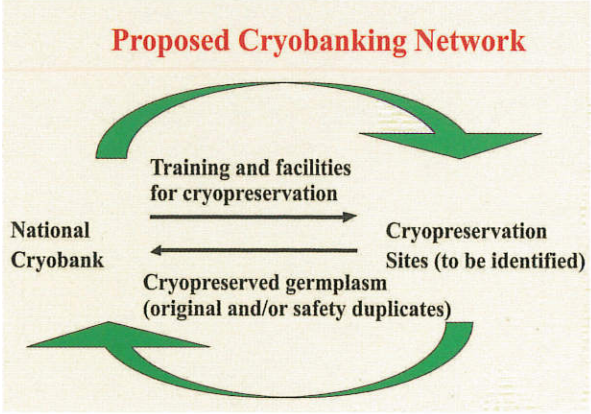
cryostorage protocols for their specific crops. A set of successfully cryopreserved germplasm in turn is deposited at the National Cryogenebank for long-term conservation. Such a networking is encouraged by NBPGR to further strengthen our efforts being made to conserve the vast diversity of plant germplasm especially producing desiccation sensitive propagules abundantly represented in India e.g. citrus, coconut, orchids, oilpalm, etc.

Achievements

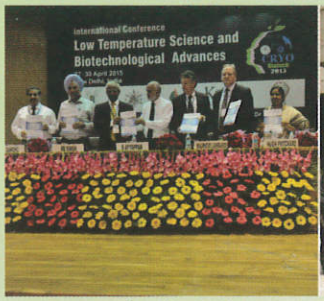
Significant progress has been made in developing cryopreservation protocols for several tropical and temperate fruits and nuts, spices, agro-forestry and forestry species, wild species, medicinal and aromatic plants. Presently more than 12,000 accs belonging to 797 species of various crop groups has been successfully cryopreserved in the form of seeds, embryos, embryonic axes, pollen, dormant buds and genomic resources. Under the HRD programme national and international trainings are also conducted in the field of cryopreservation as TCCU is designated as "Centre of Excellence" and more than 100 scientific personnel have been trained. An International Conference "Low Temperature Science and Biotechnological Advances" was successfully held in April, 2015.

Seed germination after cryopreservation in *Diospyros melanoxylon*

Healthy plantlets of *Citrus* species raised from cryopreserved embryonic axes

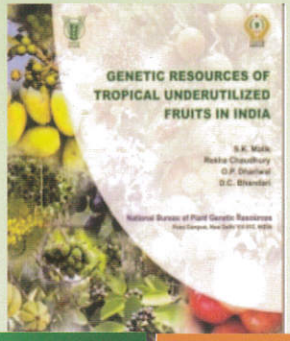


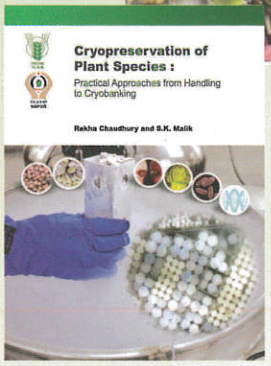
Orthodox	Non-orthodox
• Can be dried to 5%/ less MC with virtually no loss of viability	• On drying to 30% or lesser MC, viability adversely affected
• MC homogenous in seed lot	• MC varies within seed lot
• Generally small seed size	• Large seed/fruit size
• Extended life	• Life span short
• Tolerant to desiccation & freezing	• Sensitive to desiccation and freezing
• Annual crops	• Perennial trees from moist tropics, temperate & aquatic habitats
• Maturation drying	• No maturation drying
• Exhibit seed dormancy	• Metabolically active on shedding
• Fleshy coverings absent	• Seeds covered with fleshy or juicy arilloid and impermeable testa
• Desiccation sensitivity remains unaltered	• Become increasingly desiccation sensitive with storage time.

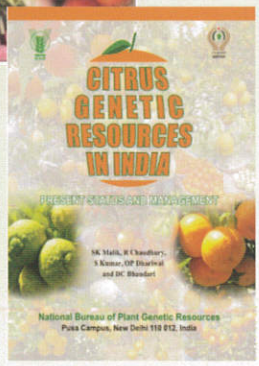


International conference

International trainees being trained at NBPGR







STATUS OF GERMPLASM AT NATIONAL CRYOBANK

(As on 31st March, 2017)

Categories	Accs (no.)
Recalcitrant & Intermediate	
Fruits & Nuts	3311
Spices & Condiments	152
Plantation Crops	22
Agroforestry & Forestry	1644
Industrial crops	1338
Medicinal species	11
Sub total	**6478**
Dormant buds	**387**
Pollen grains	**537**
Genomic Resources	**1123**
Orthodox (Medicinal spp, Wild sp., varieties, rare, endangered sp., genetic stocks)	**3795**
TOTAL	**12,320**

Total No. of species : 797

Compiled by: **Rekha Chaudhury & S.K. Malik**

Published by: ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, INDIA

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Jin-Ling Lin

NATIONAL HERBARIUM OF CULTIVATED PLANTS

National Herbarium of Cultivated Plants: A Resource for Study of Crop Genepools

Division of Plant Exploration and Germplasm Collection

ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi 110 012, India

National Herbarium of Cultivated Plants (code 'NHCP')

- **Located:** at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi; occupies a place among 25 major Indian herbaria
- **Taxa represented:** mainly of Plant Genetic Resources (PGRs) of cultivated and wild/ weedy relatives, potentially important taxa of native or introduced species for use in breeding
- **Additional material:** represented as seed/ economic products and carpological samples as complementary collections

Holdings

- **Herbarium specimens:** over 23,500 (representing 267 families, 1,521 genera and 4,271 species)
- **Seed samples:** over 3,000
- **Economic products:** over 700

NHCP

- Established in 1985 and located in a wing of the ICAR-NBPGR, Old Campus, New Delhi
- Specimens housed in specially constructed herbarium cabinets (space savers) with storage capacity upto 50,000 specimens

Allium hookeri | *Allium humile* | *Allium stracheyi* | *Allium tuberosum* | *Allium cepa* var. *aggregatum* | *Ocimum americanum*

Amaranthus tristis (=*A. tricolor*) | *Abelmoschus enbeepeegearensis* | *Populus pamirica* | *Arachis pintoi* | *Lactuca tatarica* | *Centrosema molle*

NATIONAL HERBARIUM OF CULTIVATED PLANTS

Significant Features

- **Uniqueness:** in mandate from other herbaria across the country with representation of:
  - variability in crops (as cultivars, primitive landraces, wild forms, etc.)
  - wild relatives of crop plants (CWR) from different agro-ecological habitats
- **Rare cultivars:** as herbarium/seed variability
- **Services provided:** reference collections for identification, taxonomic study; resource for teaching on plant taxonomy, ethnobotany/economic botany
- **Information resources:** scientific/technical input on identification, validation and diversity distribution in India
- **Teaching:** with Post Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi
- **Trainings:** taxonomic know-how on taxa of plant genetic resources

Significant Holdings

- ~ 350 crop plants of India under genera: *Triticum*, *Oryza*, *Abelmoschus*, *Sorghum*, *Avena*, *Coix*, *Echinochloa*, *Colocasia*, *Corchorus*, *Cucumis*, *Brassica*, *Momordica* and *Vigna*

NATIONAL HERBARIUM OF CULTIVATED PLANTS

- ~ 550 crop wild relatives (species number in parenthesis) under genera: *Oryza* (21), *Sorghum* (6), *Vigna* (25), *Cajanus/Atylosia* (10), *Allium* (29), *Abelmoschus* (15), *Solanum* (30), *Cucumis* (11), *Trichosanthes* (9), *Piper* (21), *Curcuma* (24), *Rosa* (19) and *Prunus* (16)

Active Linkages

- Forest Research Institute, Dehra Dun, Uttarakhand
- Botanical Survey of India (all regional circles)
- CSIR-NISCOM (Wealth of India) Herbarium, New Delhi
- University of Delhi, Delhi

New Records

- New taxon: *Abelmoschus tuberculatus, Herpetospermum operculatum*
- New taxa added: species under *Vigna, Abelmoschus, Cucumis*
- New recorded uses/ information: *Solena amplexicaulis, Abelmoschus manihot* var. *tetraphyllus*

Abelmoschus angulosus var. *mahendragiriensis*

Crotalaria assamica

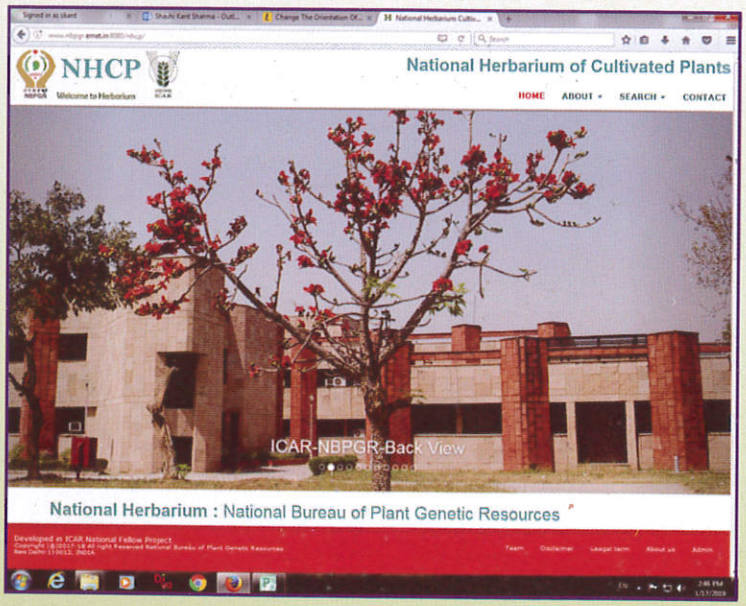
Cucumis dipsacus

Piper attenuatum

Virtual Herbarium

- Over 3,500 species of crop genepools (~7,000 images) maintained as Virtual Herbarium
- Herbarium digital resources along with data configured in web-based software in collaboration with AKMU (for exotic taxa; crop wild relatives)
- Web Linkage: https://www.nbpgr.ernet.in:8080/nhcp/

Images linked to database for quick access on a family-wise and genus-wise basis; unique identity numbers assigned to herbarium specimens (HS numbers)



NATIONAL HERBARIUM OF CULTIVATED PLANTS

Basic Studies

- Taxonomy of crop plants of Indian region
- Representation of 'difficult to process' groups
- Representation of crop cultivars, diversity/variability in PGR
- Herbarium preservation using eco-friendly methods

Facilities

- ***Herbarium laboratory*:** processing, drying, poisoning
- ***Net-house*:** grow-out of material for authentication/ validation, additions through seed material/vegetative propagules
- ***GIS lab***: geo-referencing of collected specimens
- ***Herbarium library*:** selected books/ literature
- ***Outdoor study***: field study in herbal gardens, biodiversity parks, etc.

Strengthening: The NHCP accepts unique and unrepresented genetic resources (as herbarium specimens, seed and economic products) for build-up

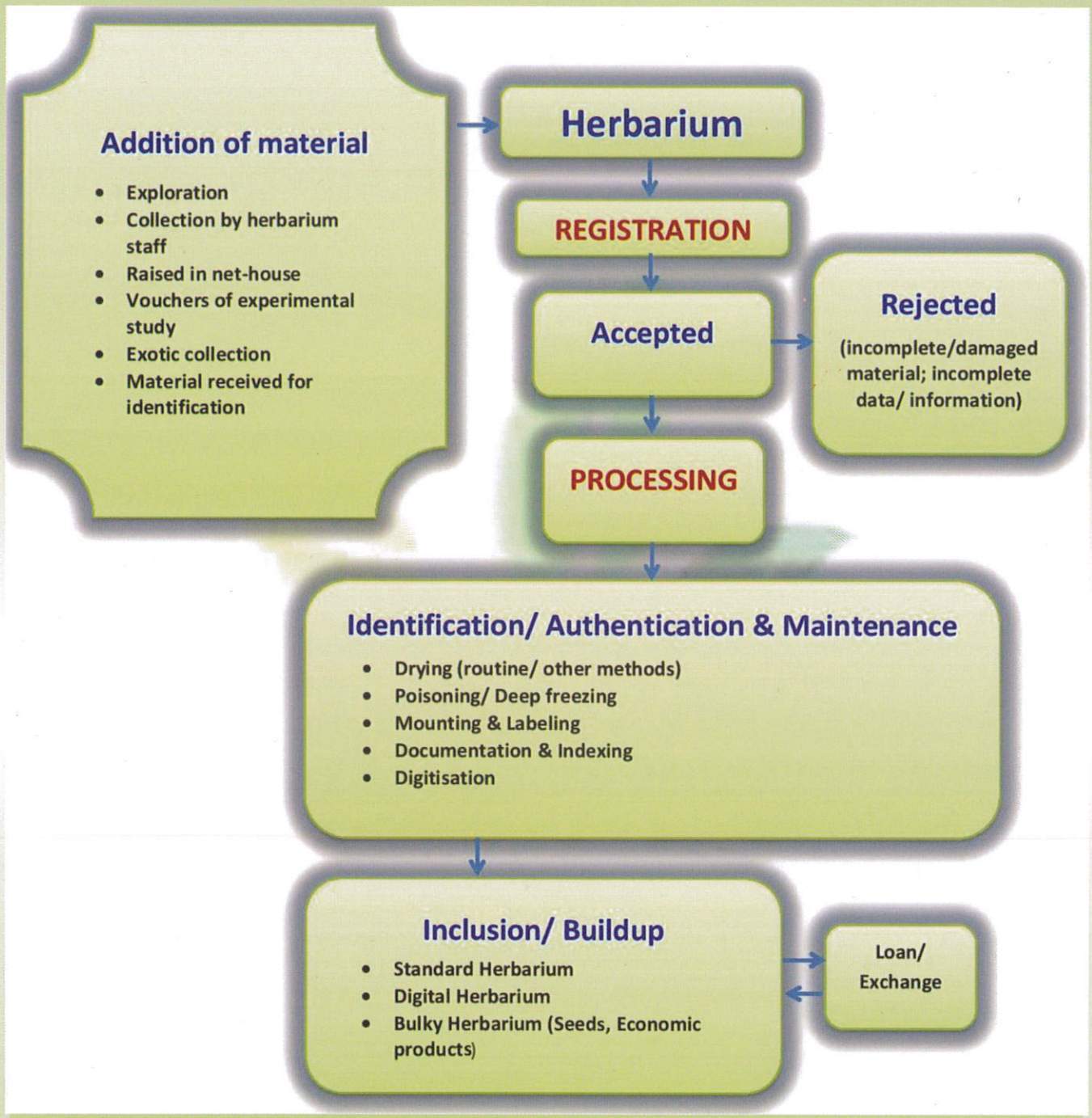
Encourages: depositing vouchers for future reference and resource for studies on PGR

Guidelines: available for use and consultation of NHCP (https://www.nbpgr.ernet.in)

ICAR-NBPGR Regional Stations Contributing to Herbarium Holdings :

- Akola, Maharashtra
- Bhowali, Uttarakhand
- Cuttack, Odisha
- Hyderabad, Andhra Pradesh
- Jodhpur, Rajasthan
- Ranchi, Jharkhand
- Umiam, Shillong, Meghalaya
- Shimla, Himachal Pradesh
- Srinagar, Jammu and Kashmir
- Thrissur, Kerala

NATIONAL HERBARIUM OF CULTIVATED PLANTS



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National Herbarium of Cultivated Plants (NHCP)

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