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Journal of Agricultural Science and Technology B 1 (2011) 975-981
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

An International Survey on State of the Art of Grain Legume Management in Gene Banks

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Received: February 22, 2011 / Published: November 20, 2011.

Abstract: An online survey addressed to members listed in the European Cooperative Programme for Crop Genetic Resources Networks Working Group on Grain Legumes and Grain Legumes (GL) germplasm managers and breeders was carried out to pinpoint the current problems in the management of GL germplasm, to work out the criteria and decisions involved in the implementation of regeneration procedures and to identify strategic areas where further research is required. The survey was divided into three sections: (1) germplasm collection details and current status of the regeneration needs; (2) assessment over the understanding of basic information required to carry out appropriate regeneration procedures such as the breeding systems, the pollination requirements and pollinating agents, the isolation techniques and regeneration facilities; and (3) assessment of different options, in addition to "*ex situ*", such as "*in situ*" and "*on farm*" conservation. Obtaining, collating and analysing different kinds of existing data on mating system of GL species, effective pollination control methods and isolation facilities by species and location is one example of a priority issue. The GL community makes a clear request for greater support for the development of well-designed methodologies of regeneration that maintain the genetic structure of populations and that the optimum regeneration strategy is most likely to be achieved through integrating pollinators with the regeneration procedures. A major concern of the GL community is the lack of empirical scientific information on the most suitable pollinator agents.

Key words: Grain legume, germplasm collections, genebanks, genetic resources management, "*ex situ*" and "*in situ*" conservation, regeneration procedures.

1. Introduction

Maintaining germplasm collections in genebanks at acceptable levels of viability and quality, demands systematic regeneration. Despite the fact of existing genebank standards and guides of crop management and regeneration, crop-specific knowledge and expertise are always required. It was recognized at the Third Meeting of the Working Group on Grain Legumes (WGGL) of the European Cooperative Programme for Crop Genetic Resources (ECP/GR)[1], Kraków, July 2001 [1] that basic information related to the development of appropriate procedures for germplasm regeneration such as the breeding system

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[1] As of 2006, European Cooperative Programme for Plant Genetic Resources (ECPGR).

and the structure and the forces that conform the genetic diversity of the land races is often lacking of allogamous legumes [2].

Additionally, this lack of information is extended to the range of complementary conservation practices such as *in situ* and on-farm that are now available [3, 4]. It may be important to provide curators and breeders with an analysis of the different management options.

It will be helpful to propose appropriate management practices to be used in "*ex-situ*" as well as "*in-situ*" and "*on-farm*" conservation strategies and accessible to a wider collective of scientists, breeders and statutory authorities associated with seed certification and purity issues.

Those considerations prompted a task force from within the membership of the ECP/GR WGGL, to organize a two-day meeting focused on key issues related to the management of Grain Legumes (GL) [5]. Key issues were identified following a review of current practices for GL germplasm management by using an online questionnaire. Analysis of the outcomes of the meeting and all the answers obtained by the online survey from the respondents has resulted in this article. By pulling together so many individual experiences and perspectives the article has to be considered by its two contributions: (1) identification of issues that have noteworthy impact on GL management and (2) a summary and analysis of respondent free comments that reveal how GL community related to the management of germplasm feels about each issue on the survey.

2. Materials and Methods

The survey was carried out in the frame work of the ECP/GR and was primarily addressed, by e-mail, to members listed in the ECP/GR WGGL and secondarily to GL germplasm managers and breeders potentially interested in the topic. The announcement of the review was web-uploaded at the AEP web page. So, anyone interested in filling the survey could do it. This online consultation was hosted by the ECP/GR Secretariat and the International Plant Genetic Resources Institute (IPGRI, now Bioversity International) website.

The survey consisted of 100 questions, the majority of which were simple, yes or no, or multiple-choice although in some of them, free comments were encouraged. The survey was divided into three sections. The first section looks at the germplasm collection details and current status of the regeneration needs. The second section tackles key questions designed to assess legume community perceptions over the understanding of basic information required to carry out appropriate regeneration procedures. Topics reviewed included: knowledge of the breeding systems, the pollination requirements and pollinating agents, the isolation techniques and regeneration facilities. Finally, the third section deals with the different options, in addition to "*ex situ*" conservation and includes questions that capture opinions and attitudes to emerging issues in the field of "*in situ*" and "*on farm*" conservation.

The questionnaire was sent via e-mail to a total number of 73 users. The amount of respondents to the survey shows that interest in the topic is rather high with 31 out of 73 answering. Responses ranged from partial completion, to complete responses and detailed free comments. The tabulated results of the survey are available at the web page of Working Group on Grain Legumes (http://www.ecpgr.cgiar.org/networks/oil_and_protein_crops/grain_legumes/previous_events.html) (verified 09/04/2011).

3. Results and Discussion

3.1 Collection Details

The activity of respondents covers nearly 180,000 entries. The global size of the collections indicates that the opinion of a great majority of GL workers on the topic have been covered in this survey. A majority of respondents define their collection more, as an active and breeder's working collection, than as a base

collection. In relation to the sizes of their collections, *Pisum* sp., *Vicia faba* and *Cicer arietinum* ranked first followed by: *Phaseolus* sp. and *Lens culinaris*. For the 5 largest species collections, between 11 and 20 curators or organizations are involved per species.

Reasons given by curators for undertaking regeneration of collections are equally distributed between the decrease of seed viability, distribution to users, seed exchanges between collections and the support of breeding activity. However, taken into account that 3 of all these aspects are interrelated most of the respondents are involved in seed multiplication to satisfy user demands and not in rejuvenation for long term storage. Frequency of regeneration by species varies considerably. In a significant number of cases, regeneration is being carried out every 5 to 9 years.

A majority of curators consider that land and space available in their respective institutions are not limiting factors for their renewal activity. Isolation tools for allogamous species and manpower for all the species represent the major limitations. As far as the number of plants per accession and the number of accessions grown in each cycle of regeneration, respondents offered a wide range of variation.

3.2 Assessment of Genetic Integrity and Breeding System

There is a necessity to clarify the general objective of regeneration [6]. Respondents were asked if it is necessary to maximize the conservation of the genetic structure of landraces. There is recognition among respondents that good regeneration procedures are integrally linked to well-designed methodologies which maximize the conservation of the genetic structure. Respondents that are not concerned about procedures that maximize the conservation of genetic structure indicated that specific regeneration procedures were conducted outside the usual work of managing the collection, e.g. as research studies. This requires considerably more resources [7].

Considering that the mating system is a key factor in the determination of the genetic structure of the diversity, respondents were asked their opinion about the following statement: “Obligate inbreeders and outbreeders are the extremes of a continuum, the probable majority of landraces will show a mixed mating with a greater or lesser tendency to self or cross pollinate between the extremes” (based on Ref. [8]). This statement achieved consent among respondents with very few exceptions. This issue has been accepted as high importance for handling accessions, because usually the mixed mating system of landraces is not taken into account. But, when it was asked, under your growing conditions how do the following species behave? A majority of respondents considered most of the species as highly inbred, i.e. almost complete selfers. There were some species (e.g., *Lathyrus sativus*, *Vicia sativa*, *Vigna unguiculata*, *Vicia narbonensis*, *Arachis hypogaea*, *Vicia ervilia*, *Medicago* sp.) in which there were disparities in opinion.

Exploring the variation in the mating system was designated as top priority for regeneration practises. The mating system can be explored in three contexts: Variation in the level of allogamy, variation in the traits that influence the level of allogamy and variation in the pollinators. First, respondents were asked if they had considered the possibility of evaluating the level of allogamy under their conditions. 30% of respondents considered that they hadn’t, it was too big a task and a majority considered that this could be a future endeavour. Regarding the methodologies for quantifying patterns of outcrossing, all responses could be sorted into two basic approaches: direct observation in terms of pollinator behaviour and pollen movement and a second approach that use genetic markers.

There were different ideas about the factors governing the variation in out-crossing but respondents have very basic knowledge about how particular plant traits influence the level of outcrossing.

Taking into account that the variation in outcrossing is the result of complex interactions of genetically controlled floral traits and pollinator behaviour [9], the understanding of how the variation on floral traits shapes the mating system is a key subject. Respondents were asked whether it is advisable to make evaluations of specific floral traits which might influence the level of allogamy. Some respondents didn't think that it was, because it would be time and resource consuming and because floral traits are not the key factor influencing the mating system. However, most respondents thought that it was. The answers offer a great range of commentaries, some of which are quoted. For instance, it was concluded that the evaluation of these traits is useful for regeneration as well as for pre-breeding and development of new genotypes by recombination. One respondent pointed out that "it is helpful, but not the whole story as pollinators plays a major role". Another one specified that it is a study they undertook with wild *Vigna* populations where autogamy and allogamy are both present. So, this highlights the need for the development of appropriate floral descriptors.

Respondents were asked their thoughts about information available about mating system variation that helps to handle germplasm. Some respondents mentioned that more information was needed for many wild species and crops with little breeding history. The majority, even considering that it is adequate, mentioned that the information tends to generalize rather than address the variability of systems operating within the genus or species and that local information on the regeneration practices in specific locations is currently lacking but would be useful to collate. All disputable points made it clear that in spite of the existence of IPGRI decision-guides, curators would like more comprehensive information than they usually manage. The guidelines should be based on practical experiences and experimental data should take into consideration geographic patterns of variation [10].

Particular future actions are (1) to collate specific data on the mating behaviour by species and location based on actual experiences, (2) to evaluate the level of allogamy by using standardized experiments and new technologies, (3) to develop a list of new floral descriptor traits. All this data on the mating system will clearly be of interest for "*ex situ*" management strategies as well as in "*in situ*" and "*on farm*" methods and will also be important when considering the management of genetically modified germplasm and for organic farming.

3.3 Assessment of Isolation Technology, Prevailing Practices and Pollinator Agents

A majority of respondents indicated that they were aware of gene flow problems and practiced some form of pollination control or isolation procedure.

Respondents were asked to specify what procedure of pollination control they used. We prepared the questionnaire providing the respondents with a list of methods commonly used in seed multiplication though they were given the opportunities of citing. Analysis of the responses showed that spatial isolation is the most common practice in the GL community. Respondents were asked about the isolation method they would recommend to others. Interestingly, the use of isolation facilities along with suitable insects as pollen vectors is the method most recommended. It was also quoted that different pollinators respond differently to plants and produce different amounts and quality of seeds.

Because of the interdependent relationship of plant flower traits and their pollinators, this raises the question of which insect species would be most effective in producing high amounts of good-quality seeds. Although few respondents have compared the efficiency of alternative methods of isolation the replies showed the following: (1) open pollination results in better quality and quantity of seeds; (2) cages without pollinators usually resulted in few seed of low quality; and (3) spatial isolation is the most

effective compared to flower or whole inflorescence bagged. Respondents were asked if it was advisable to carry out tests on the pollinator behaviour. Respondents recognize that it was. Pollinator agents, managed in the regeneration site, are effective tools for the most efficient regeneration procedures [11].

The action plan to set up is the following one: to handle pollinators as integral components in the maintenance of germplasm [12]. A basic understanding of plant-pollinator relationships in the target region is essential. This approach would help to explore such key issues as the role of the plant-pollinator relationship on shaping crop diversity and in the development of new uses of GL/GR such as sustaining wild bee pollinators [13].

A holistic approach to the management of germplasm was strongly supported among respondents. This was justified on the basis of the following three issues which were of broad appeal: it complements pollinator conservation, allows co-evolutionary interaction of pollinator-plant complexes which shape the genetic diversity and adaptability of landraces to continue and it can provide dynamic genetic pools for pre-breeding use. However, a few respondents expressed their concern about the economic cost of a holistic approach and considered that it would not be a cost-effective use of resources to achieve the goals of the genebank. A cost-benefit analysis was required that should not only focus on the short-term return of investments, but should also consider the value of (1) the preserved material and (2) associated data and information for more efficient utilization of germplasm. Moreover, respondents concurred that genebanks, apart from being seen as a means of conserving seeds for the long-term, have to be seen as a means for providing seeds for pre-breeding research. A holistic approach may therefore be helpful, not only for developing more efficient procedures of regeneration, but also for the development of pre-breeding strategies which obtain genetic materials with enhanced adaptability that at the same time show the added value of conserving biodiversity providing suitable habitats for bees [14]. Global concern on pollinator declines [15] [16] and international [17] and recent European policies [18] on sustainable agriculture could give to the opportunity for the adoption of this “biodiversity friendly” pre-breeding strategy.

3.4 Assessment of Complementary Methods to “ex situ” Conservation

The last part of the questionnaire inquires about the point of view of the respondents on complementary methods to the “*ex-situ*” conservation [19]. These questions were focused on from the practical point of view.

A majority of respondents said that there are opportunities to carry out germplasm regeneration by reintroducing landraces into local production systems. 50% of the respondents have had experiences about ways of combining static and dynamic conservation through (1) collaboration with organic farmers, (2) by re-introduction of local varieties, (3) by testing germplasm in accessible places to the local communities.

The proposal of methodologies for landrace enhancement for registration purposes is considered an important issue. More than 30% have not seen any relevant any methodology on this subject. One respondent thought that it is not reasonable, because landraces should only be a supply of useful genes. The remaining responses mentioned many different methodologies. Among them, (1) participatory plant breeding using farmers selection for the identification of landraces weaknesses which may be overcome by crossing with appropriate sources of the required traits and controlled limited gene flow, (2) soft recurrent selection methods to develop improved open-pollinated populations which integrate agronomic needs with pollinator needs, (3) evaluation and documentation of local types and their uses underlying that crop improvement needs to be closely

linked to the users, (4) One respondent suggested that it is necessary to support changes to the registration criteria because uniformity and homogeneity are not landrace's traits. This point has been addressed in some parts of Europe through derogations to EU seed legislation which allow for the registration of conservation varieties which enables the registration of landrace materials [20].

4. Conclusion

Legume genetic resources "*ex-situ*" conservation strategies have particular problems and constraints. Main points and actions emerging from the analysis of the survey are as follows.

Pre-conditions for adequate "*ex-situ*" conservation (especially regeneration) are often not met by genebanks, in order to regenerate germplasm accessions without loosing integrity. To keep genetic identity of an accession might be difficult due to very limited knowledge of the GL reproductive biology. Information on mating systems was considered too general and missing for a number of species. Moreover, there are few studies evaluating the impact of the different regeneration methodologies and their influence on the genetic structure of germplasm. More research on the mating behaviour of GL species by location based inter-disciplinary cooperation and sharing of information and responsibilities is required.

Guidelines for adequate isolation techniques/infrastructure for regeneration were considered very out of date and thus created uncertainty. In general, curators and breeders support the development of practical technical guidelines and protocols, for distribution on the web, on the use of pollinators in "*ex situ*" and "*in situ*" conservation. Collaborations between curators and breeders at an international level will certainly help to collect further evidence from research and observations by species and location related to spatial isolation.

It is essential to have a better understanding of pollinator and pollination services in conserving germplasm to obtain good (regeneration) results in "*ex situ*" conservation. There was increased recognition of importance of the adoption of holistic and multidisciplinary approaches, not limited to the classic three step approach (collection, characterization and documentation). Usually, only plant material (and related information) is collected for "*ex situ*" conservation; thus specific information on pollinator agents and plant interactions have been inadequately studied and are poorly understood. Insufficient knowledge of which pollinators to use and limited knowledge of managing pollinators and pollination is a limiting factor to some activities in genebank management. Exposure of accessions to pollinators (re-) introduces a "lost" selective influence to maintain genetic diversity in the crop. In parallel, this strategy may help to detect genotypes/populations with more positive role on pollinating insect diversity (ecological service to biodiversity offered by GL).

Legume breeders and curators were also interested to learn about and keen to further evaluate different dynamic management practices. Strategies for the conservation of genetic resources include the application of "*in situ*"/"*on farm*" measures and "*ex situ*" methods. These are complementary options to preserve the genetic resources diversity. Dynamic management of "*ex-situ*" genetic resources supplements the static conservation of seed in cold storage and needs to be promoted. Pre-breeding strategies, which allow developing pollinator-friendly improved populations, should be available for on-farm conservation and participatory breeding.

Acknowledgments

The survey on Grain Legume Management in genebanks has not been possible without the help of many people. The task force would like to thank: Lorenzo Maggioni for his support, also special thanks to Aixa Del Greco for her continuing assistance and Massimo Buonaiuto for performing the analysis of the questionnaire. In addition, thanks to all the

respondents for dedicating their time to complete the survey and for providing an interesting data set. The survey was hosted by the ECP/GR Secretariat and IPGRI website.

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