Ricebean Network

Project no. **032055**

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Project title: **Food security through ricebean research in India and Nepal**

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Project coordinator name: Dr PA Hollington
Project coordinator organisation name: CAZS Natural Resources, University of Wales Bangor

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Abstract

The project Food Security through Ricebean Research in India and Nepal (FOSRIN) is a three-year STRP funded by the 6th Framework Programme of DG Research of the European Commission under the International Cooperation (INCO) Programme. This document summarises the activities that took place within FOSRIN in the period 1 April 2007 – 31 March 2008. Achievements and work progress are described for all relevant workpackages, and additional information is provided on project management, coordination activities, and dissemination and exploitation of results. The document contains a 4-page *Publishable Executive Summary*, and as an annexe the current version of the *Plan for Using and Disseminating the Knowledge*. The document has been edited prior to publicationon the website.

Ricebean is an underutilised grain legume, grown by hill farmers on marginal land particularly in Nepal and northern India. The project aims to promote the crop and widen its use through a number of activities. Activities include assessment of the supply chain to see where value may be lost; the assessment of genetic diversity and indigenous knowledge of the crop, and the assessment of the potential impact of enhanced pulse availability on local human nutrition. We aim to develop a Market-based Legumes Traits Value-Index (MLTVI) that allows breeders to assess legumes in terms of their monetary value to consumers, and innovative and efficient marketing methods for high quality, protein-rich products. Finally, we will develop policies to support and promote equitable access to such protein-rich foods and so build sustainable medium and long term food security

During the reporting period, three contracted deliverables were produced: documents on the distribution of ricebean in India and Nepal (Deliverable 2.1); on the identification of polymorphic markers (Deliverable 3.1); and on indigenous knowledge if ricebean in Nepal (Deliverable 2.2). In addition, annexes with detailed information on experimental protocols, germplasm characterisation, and SSR primers screened, are provided.

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Executive summary

Introduction. This document describes the second year's work of the FOSRIN (**Fo**od **S**ecurity through **R**icebean Research in **I**ndia and **N**epal) project. FOSRIN is a consortium of eight partners, universities, NGOs and government research organisations in Europe and South Asia, working to popularise the underutilised grain legume crop ricebean (*Vigna umbellata*) and promote its cultivation over a wider area of the environments to which it is suited than is currently the case. The work involves research on the supply chain and marketing of the crop, the diversity and adaptation of germplasm, farmers' preferred traits and indigenous knowledge of the crop, and its health and nutritional aspects. The project, funded by DG Research of the European Commission under the 6th Framework Programme (FP6) began in April 2006, and continues until March 2009. The work of the project is showcased on the website of The Ricebean Network (www.ricebean.org).

Background. Production of cereals in South Asia has far outstripped the production of legumes, with serious consequences for the food security and nutritional well-being of poor farmers in marginal areas. Ricebean is a grain legume grown in Western, Northern and Eastern (WNE) India and Nepal. It is widely grown as an intercrop, particularly of maize, and was widely grown in the past on residual water after rice. There is little or no choice of improved varieties as there has been almost no modern plant breeding, landraces predominate and seed supply is limited or non-existent. Consequently, it is not grown widely despite its suitability for marginal agricultural areas where many poor people live. Ricebean grows well on a range of soils. It has rapid establishment, is pest resistant, and has the potential to produce large amounts of nutritious animal fodder and high quality grain, and there is great scope for genetic improvement in this neglected crop.

Objectives. The overall objective is to make ricebean more than locally popular by identifying and measuring the diversity within the range of germplasm available in India and Nepal and characterising it for suitability to the cropping systems of the region. We will match farmer-preferred varieties to diverse seasons, environments and markets, using a combination of genetic, agronomic, and socio-economic approaches and using client-orientated principles to identify genotypes and parents for breeding programmes suitable for integrating ricebean into rice- and maize-based cropping systems in WNE India and Nepal. Our specific scientific objectives are as follows:

1. To analyze the legumes supply-chain for stages and linkages where product value of improved ricebean is potentially lost or where information on product quality may be compromised or lost
2. To assess genetic diversity and indigenous knowledge on ricebean in Nepal and India
    2.1. To assess genetic diversity and uses of ricebean using indigenous knowledge of the crop
    2.2. To characterise the germplasm diversity using molecular marker techniques
    2.3. To characterise the germplasm for phenological traits and suitability for a range of diverse environments and cropping systems using participatory approaches
3. To assess the potential impact of enhanced pulse availability on local human nutrition
4. To develop a Market-based Legumes Traits Value-Index (MLTVI) that allows breeders to assess *ex ante* the value of new legumes in terms of their monetary value to consumers
5. To develop innovative and efficient marketing methods for high quality, protein-rich products from the crops to increase market accessibility, product value and promote export value

6. To develop policies to support and promote equitable access to such protein-rich foods, building sustainable medium and long term food security

In addition, we have 3 management objectives to ensure the smooth running of the project: these are detailed in the full report.

Contractors involved and coordinator contact details

Contractors	Senior staff involved
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Dept. of Agricultural Economics, Christian Albrechts University of Kiel, Germany (CAU):	Prof Dr Rolf Mueller, Prof Dr M von Oppen; Ms Doreen Buergelt
Department of Geography, University of Bergen, Norway (UB)	Dr Peter Andersen
Crop Research Component, Gramin Vikas Trust, Dahod, Gujarat, India (GVT)	Dr JP Yadavendra
Ch. Sarwan Kumar Krishi Vishwa Vidyalaya**,** Palampur, Himachal Pradesh, India (CSKHPKV)	Dr Naveen Kumar, Dr JC Bhandari, Dr RK Chahota, Dr DK Banyal, Dr Harbans Lal, Dr Rajan Katocht
Assam Agricultural University, Jorhat, Assam, India (AAU)	Dr Mrs SB Neog
Nepal Agricultural Research Council, Kathmandu, Nepal (NARC)	Dr Mrs Jwala Bajracharya, Mr Man B Shrestha
Local Initiatives for Biodiversity, Research and Development, Kaski, Nepal (LI-BIRD)	Dr Pratap K Shrestha, Mr Resham Gautam

Work performed and results achieved

To meet the scientific objectives, we developed five workpackages. The first was concerned with the marketing and supply chain of the crop. After a literature review, a working paper on approaches to supply chain analysis for pulses in India and Nepal has been drafted. Intensive surveying of ricebean markets, and sampling, was carried out in India and Nepal at different stages of the supply chain. To develop the MLTVI, laboratory tests have been devised after consultation with experts, and used to measure traits in ricebean and other pulses. We analysed these samples to assess differences, in particular to compare ricebean to chickpea, the most common pulse in the region. The analysis included both physical and nutritional parameters. Of the pulses analysed, ricebean had the lowest water uptake capacity (and highest moisture content), the highest ash and protein contents, and the lowest fat.


Ricebean flowers at the evaluation trial in Karbi Along, Assam (Mr R Gautam, LI-BIRD)

The second workpackage was on assessing genetic diversity and indigenous knowledge. Germplasm collection continued in 2007 as an adjunct to other activities – in future we aim to record GPS coordinates of collection sites. Evaluation was at 2 field sites in Nepal, Darbar Devisthan and Simichaur VDC, Gulmi district, Western Nepal. These had an average altitude of 1350 m asl, and were dominated by sloping *Bari* land.

The core collection of 50 accessions, with 2 replications, was grown as an intercrop with maize at Gulmi, while 66 non-core accessions were grown as a sole crop also at Gulmi. Principal components analysis was used to group the material. In India, germplasm evaluation was carried out at one location in Assam, one in Himachal Pradesh, and two in Madhya Pradesh (MP). A total of 23, 66 and 15 genotypes respectively were evaluated.

We designed, reviewed and refined the documentation for the indigenous technical knowledge (ITK) work, and developed and refined a checklist. The first round of documentation was completed in Gulmi, and activities initiated in Ramechhap. In India, documentation of ITK has been carried out in Palampur, with the emphasis on recipes and culinary uses. Photographs of ricebean preparations in the area are available on the website.

In WP3, work on molecular diversity of ricebean was continued by NARC. Two hundred and eighteen accessions of ricebean collected across Nepal were studied for agro-morphological and molecular diversity to detect the extent of genetic diversity. Experiments on core and non-core collections were carried out in four agro-ecosystems: Rampur, (sub-tropical), Khumaltar (mid-hill valley), Gulmi (mid-hill) and Kabre (high-hill. Diversity for agro-morphological traits for both collections was very high. Based on this and on field performance, ten accessions from the core, and 21 from the non-core set, were selected for further evaluation. By screening 109 azuki bean SSR primers over 27 stratified ricebean samples, a set of 49 primers were found to amplify ricebean DNA. Among these 35 were polymorphic, with an average 0.24 PIC between ricebean accessions, of which 13 primers with higher than average polymorphism could be used to detect genetic diversity in ricebean. The primers were of di-nucleotides of AG, distributed across the genome, and will be used for the molecular marker diversity analysis of ricebean landraces in 2008/09.

WP4, on germplasm characterisation, was led by the Indian NGO GVT, with inputs from the other Asian partners. The 103 germplasm lines collected last year, were evaluated in *Kharif* 2007 for their quantitative characters by all the Indian partners in farmers' fields to identify promising lines suitable for cultivation in the region. Over 50 more lines were collected from different areas in *rabi* (winter) 2007/08 from household stores on farms.

Mother and baby trials were conducted on a large scale. Data on yield and yield attributes were recorded and analysed. Matrix ranking of farmer preferences was done in mother trials, and was also recorded in the baby trials. Most farmers preferred short duration, bold seeded varieties. The baby trials revealed a clear preference for bold seeded varieties with determinate growth, early to medium maturity, tolerant to drought and low shattering giving more grain yield and a plant type suited for growing as an inter or mixed crop with maize, sorghum or on terrace risers. However, getting some of the organoleptic traits from preferred existing landraces should strengthen the role of ricebean in local farming systems.

An attempt was made to assess the performance of ricebean planted during winter in hill areas of peninsula India. Germination and plant population was good at all locations but due to low temperature there was no fruit set observed.

Nuggets – ricebean *dhal* soaked, ground and prepared in various ways (Dr Naveen Kumar, CSKHPKV)

UB were responsible for WP5, on health and nutritional aspects of the crop. A questionnaire and standard food models were developed, and surveys carried out on three aspects at three periods over six months in 2007, in 6 areas in Nepal and two States in India. The information will identify nutritional values and draw out the general nutritional structure in villages with and without ricebean in their diets. The data have been formatted for the WorldFood2 programme, and a full range of nutrient figures has been compiled in WorldFood2 format, mostly from existing literature.

Ricebean has a moderate raw protein content but with high digestibility and a very beneficial amino acid composition. Its vitamin and mineral content is comparable to other pulses, and it has a low fat content. There are no specific problems concerning toxic or allergenic substances, and no unusual anti-nutrients. However, the low levels of the crop in diets mean there is no significant benefit in terms of these nutrients at present.

In India, a range of recipes have been tested with consumers to try to develop value-added products. Of ten basic products, there was keen interest shown in nine, supporting the view that there is a potential market for value-added products from ricebean. Further organoleptic evaluations will be carried out over the coming year.

Expected end results

We anticipate that by the end of the project we will have developed a hedonic demand function for legumes and completed and quantified the MLTVI, as well as designed a strategy for the introduction of ricebean into the legumes supply-chain in India and Nepal. We will have an understanding of ricebean's distribution in India and Nepal, of its diversity in terms of farmers' names, of phenology and morphology, and on a molecular basis, and of the indigenous technical knowledge on the crop's production and use. We will also have a workable set of polymorphic markers for the crop which may also be of use to workers on other *Vigna* species. We will know the traits that farmers regard as being important, and have identified particular varieties that meet those requirements. Finally, we will understand the potential impact upon human health and nutrition that the introduction of ricebean into the diets of a larger proportion of the population could have.

Intentions for use and impact

The results of the project will be of interest to a wide range of audiences. These include scientists working in project-related areas, including on other grain legumes, as well as farmers and farmers' organisations in the region, market traders, intermediaries and their organisations, urban and rural consumers, and policy makers.

It is our intention that farmers in the region will adopt the new technologies and germplasm developed by the project. Based upon our previous experience, we expect this to be as much by informal farmer-to-farmer dissemination as by the extension activities of the project partners. This will have a direct impact in terms of improving the farmers' livelihoods and food security through increased use of legumes with inherent abiotic stress resistance, high biomass production and good nutritional quality that would obtain a good market price. Improved fodder production will improve livestock health and production, and greater use of legumes in the farming system will reduce erosion, fix nitrogen, and increase soil organic matter. By illustrating the value of research into underutilised crops we will impact on policy, and we will improve equity by targeting the results at resource-poor farmers. We also believe that in the longer term the knowledge generated by FOSRIN will greatly assist the breeding of improved and well-adapted varieties of ricebean. The development in particular of the MLTVI will provide an exceptionally useful tool for plant breeders, not only of ricebean but also of similar grain legumes in S Asia, by enabling them to allocate a monetary market value to the traits they are breeding or selecting for.

Main elements of the publishable results of the plan for using and disseminating the knowledge

As noted in the year 1 report, a comprehensive ricebean bibliography is available on the project website at http://www.ricebean.org/references1.htm. Three deliverables have been produced: documents on the distribution of ricebean in India and Nepal (Deliverable 2.1); on the identification of polymorphic markers (Deliverable 3.1); and on indigenous knowledge if ricebean in Nepal (Deliverable 2.2).

Section 1 – Project objectives and major achievements during the reporting period

1.1 Overview of general project objectives and current relation to the state-of-the-art

Introduction. Cereal production in S. Asia has far outstripped that of legumes, with serious consequences for the food security and nutritional well-being of poor farmers in marginal areas. Ricebean (*Vigna umbellata*) is a legume grown in Western, Northern and Eastern (WNE) India and Nepal. It is widely grown as an intercrop, particularly of maize[1], and was often grown in the past on residual water after rice. There is little or no choice of improved varieties as there has been almost no modern plant breeding. Landraces predominate and seed supply is limited or non-existent. Consequently, it is not grown widely despite its suitability for marginal agricultural areas where many poor people live. Moreover, well-functioning marketing channels for the crop do not exist. Ricebean grows well on a range of soils. It has rapid establishment, is pest resistant, and has the potential to produce large amounts of nutritious animal fodder and high quality grain, and there is great scope for genetic improvement in this neglected crop.

Objectives. The overall objective is to make ricebean more than locally popular by identifying and measuring the diversity within the germplasm available in India and Nepal and characterising it for suitability to the cropping systems of the region, matching farmer-preferred varieties to diverse seasons, environments and markets, using a combination of genetic, agronomic, and socio-economic approaches firmly based on client-orientated principles to identify genotypes and parents for breeding programmes suitable for integrating ricebean into rice- and maize-based cropping systems as well as into the diets of consumers in WNE India and Nepal.

The scientific objectives of the project are as follows:

1. To analyze the supply-chain for stages and linkages where product value of improved ricebean is potentially lost or where information on product quality may be compromised or lost
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In addition, we also aim:

7. To ensure effective integration of results, hypotheses and germplasm, and their wide dissemination to stakeholders and other interested parties
8. To ensure dialogue between participating institutions, research teams, other projects, participating communities and governments
9. To strengthen sustainably the research capability of the Asia Partner Country institutes involved in the project

[1] Lawn, RJ (1995)

Current relation to state-of-the-art

The sustainability of continuous cereal systems is in doubt unless broken by a legume. However, in Northern and Eastern hill areas of India and in Nepal, farmers have only a limited choice, usually greengram (*Vigna radiata*) or blackgram (*V. mungo*). Neither is ideal due to their high water use and long duration. Farmers' preliminary consultations suggest that they need a short duration legume, with few disease and pest problems. Ricebean has all the required traits except grain quality and photoperiod-insensitivity. It grows well on a wide range of soils, and has good pest resistance. Some genotypes are drought tolerant, and the twining habit of some genotypes makes it suitable to intercrop with maize, although difficult to harvest mechanically. With its quick growth and good biomass production it can be used as animal feed during the fodder-scarce summer, and as a green manure.

Germplasm and indigenous technical knowledge. There has been no systematic attempt to collect Nepalese ricebean germplasm, very few studies on its diversity, and no systematic documentation of indigenous technical knowledge on the crop, and little in India since the 1960s, although indigenous and exotic collections evaluated in the 1970s[2] showed a wide range of genetic variation for morpho-agronomic attributes. Existing indigenous knowledge on ricebean in both countries needs documenting and collating. We have previously developed participatory methods to systematically collect germplasm and relate it to various socio-economic questions, so germplasm evaluation will not rely on field evaluation or molecular marker diversity alone, but be supported by these other approaches to allow a better understanding of genetic diversity.

Farmers' knowledge has been most commonly and widely discussed using the term *indigenous knowledge*'[3] a label that some major institutions working on the subject have adopted. However, the great diversity of disciplines in both the natural and the social sciences that have been involved in this, as well as the value judgements of individual investigators, has led to the concept been described and discussed using a large number of terms: these are detailed in Deliverable 2.2[4].

While these definitions may in many contexts be useful, they serve to seriously constrain how local knowledge can be gathered and used. Although the way in which local knowledge is acquired and transformed into decisions depends on the cultural context, knowledge is distinguishable from other aspects of a person's, or a community's culture. A 'utilitarian' approach to the definition and use of local knowledge in research and development defines knowledge as 'the outcome, independently of the interpreter, of the interpretation of data, that can be articulated and communicated', and local knowledge as 'locally derived understanding which is based on experiences and real world observation.[5]'

The central tenet of Participatory Technology Development (PTD) is to facilitate and support farmer experimentation by combining farmers' local knowledge and methods with advances in scientific knowledge and methods. Despite a growing interest and emphasis on local knowledge, its use in research and development has been constrained by a lack of appropriate methods for storage, analysis, synthesis and interpretation of the qualitative knowledge held by farmers[5].

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[2] Chandel, KPS *et al* (1988). Rice bean - a potential grain legume. NBPGR Sci. Monogr. No. 12. NBPGR, New Delhi

[3] Thapa, B *et al* (1995). Incorporation of indigenous knowledge and perspectives in agroforesty development. II: Case study on the impact of explicit representation of farmers' knowledge. Agrofor Sys **30**:249-261

[4] Khanal, AR & Poudel, I (2008) Farmers' local knowledge associated with production, utilization and diversity of ricebean (*Vigna umbellata*) in ricebean growing areas of Nepal. Deliverable 3.1, FOSRIN Project. Pokhara, Nepal: LI-BIRD.

[5] Sinclair, FL & Walker, DH, (1999). A utilitarian approach to the incorporation of local knowledge in agroforesty research and extension. In: Shrestha PK (2003) Incorporating local knowledge in participatory development of soil and water management interventions in the Middle Hills of Nepal, PhD thesis, Univ. Wales, Bangor, UK.

Farmers' decisions whether to select, reject or maintain a particular landrace at any given time are influenced by many environmental, biological, cultural and socio-economic factors[6]. Understanding farmers' practices, and the underlying local knowledge regarding them, is useful to guide the conservation and promotion strategy for any indigenous crop. As farmers have been cultivating ricebean for many generations, they have unique practical knowledge of the crop, and an assessment of this is an essential prelude to the promotion of this underutilized crop.

The use of farmers' local knowledge in research and development requires a methodological framework that allows it to be effectively and systematically stored, accessed, analyzed, and synthesized, so making it available for future use[7]. Knowledge-based systems (KBS) developed originally for agroforestry research and extension have been successfully used in Nepal, especially in the collection and analysis of farmers' ecological knowledge about tree fodders[8]. Such systems are increasingly being used in a number of countries worldwide in a variety of disciplines.

Farmers' perceptions and knowledge of diversity. Farmers grow landraces to meet their agronomic and cultural needs, and have a well-developed indigenous knowledge of their crops and varieties. Farmers' diversity management consist of seed flows, variety selection and adaptation, and seed selection and storage[9], all influenced by agro-ecological, socio-economic and cultural conditions, many of which affect the management of landrace diversity, so farmers' indigenous knowledge is linked to the maintenance and management of genetic diversity[10,11]. Landrace choice is primarily determined by adaptation to the agro-ecological domain and farm management practices, followed by selection for phenotypic features that best meet farmers' preferences.

Analysis of genetic diversity using morphological and molecular data. Traditionally, legume variability is largely described by their morphology, agronomic behaviour, and on biochemical traits. It is generally associated with a low level of diversity[12,13]. However, variability in Asian *Vigna* has more recently been studied using a variety of molecular techniques as well as traditional agro-morphological characterisation. Restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNAs (RAPDs), inter simple sequence repeats (ISSRs), and microsatellites or simple sequence repeats (SSRs) are molecular marker techniques that have been extensively used in genome analysis of the Asian *Vigna*, especially Adzuki bean (*V. angularis*). Using recombinant DNA technology, variation in DNA sequences can be examined directly, avoiding environmental effects which could confound morphological evaluation, and possibly biased allozyme estimates.

Recent work has assessed between-farm diversity in cowpea (*V. unguiculata*) landraces using AFLP and SAMPL markers to determine the distribution of genetic variation[14], genetic

[6] Bajracharya, J *et al* (1999). Farmers selection of germplasm using agromorphological and isozyme characteristics. A scientific basis of *in-situ* conservation of agro-biodiversity on-farm: Nepal's contribution to the Global Project .NP Working Paper No.1/99. NARC/LI-BIRD, Nepal/IPGRI, Rome, Italy

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[9] Bellon, MR *et al* (1997) Genetic conservation: a role for rice farmers. In Maxted BV *et al* (eds) Plant genetic conservation: an *in-situ* approach. London, Chapman Hall

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[13] Lumpkin TA & McClary DC (1994). *Adzuki bean: Botany, production and uses.* CAB International, Wallingford, UK.

[14] Tosti, N & Negri, V (2005) On-going on-farm microevolutionary processes in neighbouring cowpea landraces revealed by molecular markers. *Theor Appl Genet* **110**: 1275-1283

diversity in blackgram has been studied using RAPD and ISSR markers[15], and in greengram using AFLPs[16]. Molecular markers have been used to study genetic diversity in rice landraces in Nepal[17]. Twelve *Vigna* microsatellites were identified earlier[18], and an integrated consensus map developed for cowpea, containing over 400 markers[19].

Linkage maps have been developed for three of the Asian *Vigna* species: mung bean (*V. radiata*), adzuki bean and black gram[20,21] and a large number of SSR markers have been developed for adzuki bean[22,23]. These have been used in comparative linkage maps in other related legumes and have provided information on genetic relationships among the related species. However, no molecular information is yet available for ricebean.

Germplasm characterisation – participatory methods. Germplasm characterisation using agromorphological and molecular data is valuable for breeding programmes, but as well as testing across environments complete evaluation additionally requires measures of farmers' preferences for varieties and traits. Well established participatory methods for variety evaluation in farmers' fields often use single replicate, multi-entry trials (mother trials) and single intervention trials of a new entry versus a local check (baby trials)[24]. Mother trials sample more environments than replicated on-station trials[25], and baby trials allow the cost-effective use of many replicates[26], contributing major improvements over more conventional varietal testing:

(1) Participatory trials allocate more resources to more advanced lines than many conventional breeding programmes[27].

(2) Farmers can evaluate varieties for all traits and make trade-offs of, e.g., grain yield against fodder yield, maturity, and grain quality.

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[17] Bajracharya, J (2003) Genetic diversity study in landraces of rice (*Oryza sativa* L) by agromorphological characters and microsatellite DNA markers. PhD Thesis, Univ. Wales, Bangor, UK

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(3) Varieties are tested under realistic management, across more physical niches as trials are replicated in more locations, and also across social niches where food preferences might vary.

Although these, or similar, approaches have been applied to several crops, no work has been done on ricebean.

Market requirements. Crop breeding adds economic value in two ways, by lowering the costs of produce, and by adding value through improved cooking and keeping qualities. Conventional breeding focuses on crop traits that reduce production costs or storage losses, or both, but traits that add value or reduce costs at the household level are usually ignored for lack of information. Current economic demand theory and econometric methodology added to experience from applied studies allows us to develop an index to guide breeders towards adding value by satisfying consumer wants[28]. Ricebean is mostly produced for subsistence and it is not well introduced into supply-chains, but without market sales the benefits of improved varieties would not reach urban consumers. Developing markets for improved ricebean will be based on an analysis of existing legumes markets, and will require mechanisms that assure both the flow of the material product and of product information from producers to consumers. The information flow requires research, as some quality characteristics valued by consumers are likely to be invisible and will not be automatically passed on along the supply-chain, so must be communicated by other means. Whenever information about quality characteristics is separate from the good that has the characteristics this information may be lost, misrepresented, or otherwise become corrupted, so that buyers' valuation of improved ricebean products will be reduced. Also, if ricebean's true quality is misrepresented consumers' willingness to pay for improved varieties will be reduced.

Health and nutrition. Average legume consumption in Nepal is below suggested FAO levels[29], and in India only just reaches them[30]. Pulses are expensive for poor people, and farmers' yields are low. As pulses have only been replaced to a small extent by animal source foods, there has been a strong decrease in micronutrient density in the diet, and a steady rise in the proportion of people suffering from anaemia and other deficiencies[31], with around 95% of the population of S. Asia at risk of zinc deficiency[32]. Food-based strategies are of specific interest in poor populations, especially allied to increasing production, profitability and sustainability of smallholder agriculture. Fortifying food products with protein-rich ricebean flour can improve diets, so expanded ricebean consumption in marginal areas could increase access to food with high protein and essential mineral content, but its reputation as a food for the poor may hinder to its spread. One factor limiting the spread of the positive effects of the Green Revolution among many of the world's poorest rural communities is lack of recognition of inter-farm heterogeneity[33], which requires similar heterogeneity in the innovations provided.

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1.2 Summarise the objectives for the reporting period, work performed, contractors involved and the main achievements in the period

Table 1.1: Workpackages and WP leaders

WP No.	WP Title	WP Leader	Objective
Scientific			
1	Supply chain, demand and marketing	CAU	1, 4, 5, 6, 9
2	Genetic diversity and indigenous knowledge	LI-BIRD	2, 9
3	Molecular markers	NARC	2, 9
4	Germplasm characterisation and adaptation	GVT	2, 9
5	Nutrition and health	UB	3, 5, 6, 9
Management			
6	Coordination and management	CAZS-NR	7, 8, 9
7	Dissemination	CAZS-NR	7, 9
8	Review	CAZS-NR	

To meet the project objectives, we are carrying out 5 scientific and 3 management workpackages, as noted in Table 1.1. Work was carried out in all of these to meet the specific objectives for the second reporting period, April 1, 2007 – March 31, 2008 , which were as noted in Table 1.2. The timing of these milestones was altered substantially from that foreseen in Annexe 1 due to delays in signing the contract with the consequent effect on the relationship of the project to the crop calendar, although some work was able to take place before the contract was signed. Further slight alterations were made at the 2nd Annual Meeting as a result of experimental problems, as well as problems resulting from political difficulties in Nepal. An updated, frontlined barchart is appended in Section 3.

Table 1.2: Objectives for the second reporting period

Objective	WP	Partner	Month*
To review literature on the ricebean supply chain and test the proposed methodology	1	CAU	12 (7)
To complete a survey of ricebean diversity in the study sites	2	LI-BIRD	12 (10)
To document national distribution of ricebean	2	LI-BIRD	15 (3)
To complete the field surveys in India and Nepal	5	UB	18 (4)
To hold the 2nd Annual Meeting	6	CAZS-NR	18 (12)
To identify polymorphic DNA markers	3	NARC	18 (12)
To complete field evaluations of germplasm	2	LI-BIRD	18 (18)
To complete the first year of mother and baby trials	4	GVT	21 (12)
To complete field research in Asia	2	CAU	24 (18)
To report on dietary patterns and nutrition	5	UB	24 (10)

*Revised. Initial month foreseen in parentheses.

Work in WP 1

This work is led by CAU Kiel, with assistance from the Asian partners. After a literature review, a working paper on approaches to supply chain analysis for pulses in India and Nepal has been drafted. Intensive surveying of ricebean markets, and sampling, was carried out in India and Nepal at different stages of the supply chain. To develop the MLTVI, a set of laboratory tests has been devised after consultation with experts, and used to measure a number of traits in ricebean and other pulses. We analysed these samples to assess their differences, in particular to compare ricebean to chickpea, the most common pulse in the region. The analysis included both physical and nutritional parameters. Of the pulses analysed, ricebean had the lowest water uptake capacity (and highest moisture content), the highest ash and protein contents, and the lowest fat.

Work in WP2

Germplasm collection continued in 2007 as an adjunct to other activities – in future we will record GPS coordinates of collection sites where possible. In 2007 evaluation trials were conducted at two field sites in Nepal by LI-BIRD, in Darbar Devisthan and Simichaur VDC, Gulmi district, Western Nepal. These had an average altitude of 1350 m asl, and were dominated by sloping *Bari* land. The core collection of 50 accessions, with 2 replications, was being grown as an intercrop with maize at Gulmi, while 66 non-core accessions were being grown as a sole crop also at Gulmi. Additional data will be collected at and after harvest. Principal components analysis was used to group the material. In India, germplasm evaluation was carried out at one location in Assam, one in Himachal Pradesh, and two in Madhya Pradesh (MP). A total of 23, 66 and 15 genotypes respectively were evaluated.

We designed, reviewed and refined the documentation for the indigenous technical knowledge (ITK) work, and developed and refined a checklist. The first round of documentation has been completed in Gulmi, and activities initiated in Ramechhap. In Gulmi, 15 interviews have been carried out, stratified by gender, ethnicity and altitude. In India, documentation of ITK has been carried out in Palampur, with the emphasis on recipes and culinary uses. Photographs of ricebean preparations in the area are available on the website.

Work in WP3

This work is carried out by NARC, with back-up and assistance from CAZS-NR. Two hundred and eighteen accessions of ricebean collected across Nepal were studied in 2007 for agro-morphological and molecular diversity to detect the extent of genetic diversity in ricebean germplasm. The study comprised two field experiments: a core evaluation of 50 and a non-core evaluation of 168 accessions. Both experiments were carried out in four agro-ecosystems: Rampur, (sub-tropical), Khumaltar (mid-hill valley), Gulmi (mid-hill) and Kabre (high-hill. Diversity for agro-morphological traits for both the core and non-core collections was very high, ranging from 0.16 to 0.48 CV and 0.43 to 0.93 diversity index. Based on observed diversity and field performance, ten accessions from the core, and 21 from the non-core set, were selected for further evaluation in 2008. After screening simple sequence repeat (SSR) primers over 27 stratified ricebean samples, a set of 35 adzuki bean SSR primers were found to amplify ricebean DNA. Among these amplified primers only 13 were found to be polymorphic, with an average 0.24 polymorphic information content (PIC) between the ricebean accessions. These could be used to detect diversity in Nepalese ricebean germplasm. These primers were distributed across the genome and constituted di-nucleotides of AG, and will be used for the molecular marker diversity analysis of ricebean landraces in 2008/09.

Work in WP4

This WP is led by GVT, with inputs from the other Asian partners. The 103 germplasm lines collected last year, were evaluated in *Kharif* 2007 (rainy season) for their quantitative characters by all the Indian partners at their respective locations. The germplasm lines were evaluated and characterized under farmer’s field situation for isolation of the promising lines suitable for cultivation in the region. 54 more lines have been collected from different areas of the country during winter season of 2007/08 from the stores of farmers in their households.

Mother and baby trials were conducted on a large scale in the project area. The data on yield and yield attributes were recorded and analysed. Matrix ranking was done in mother trials on the basis of farmers’ preferences, while in the baby trials farmers’ preference were also recorded. Most farmers preferred short duration and bold seeded varieties of ricebean. The baby trials revealed that farmers have a clear preference for bold seeded varieties with determinate growth, early to medium maturity, tolerant to drought and low shattering giving more grain yield and a plant type suited for growing as an inter or mixed crop with maize, sorghum or on terrace risers. However, getting some of the organoleptic traits of the most preferred existing landraces will probably help strengthen the role of ricebean in the farming systems.

An attempt was made to assess the performance of ricebean if planted during winter season in the hilly tract of Jhabua region as well as in eastern Gujarat. The germination and plant population was good at all the locations but due to low temperature only vegetative growth was seen, no fruit set was observed. An experiment on seed priming of ricebean was also conducted at CSKHPAU Palampur

Work in WP5

A questionnaire and standard food models were developed, and a survey carried out on three aspects at three periods over six months in 2007, in 6 areas in Nepal and two States in India.

The survey was carried out in 100 households in each district, using women in the 25-45 age group. The information is being used to identify nutritional values and status of these groups to draw the general nutritional structure in villages of Nepal with and without ricebean in their dietary system. The data have been formatted for the WorldFood2 standard programme. The analysis is expected to be carried out over the following months, a slight delay compared to the original plan, but not putting the deliverables from WP5 at any risk.

With the aim of analysis of the specific nutritional value of ricebean, a full range of nutrient figures has also been compiled in WorldFood2 format. Most values have been compiled from existing literature and, due to geographic variation in the sources, and the variability of ricebean, have been selected according to what appears to be consensus, from what can be assumed as the best, peer reviewed literature. Information obtained so far shows that ricebean has a moderate raw protein content but with high digestibility and a very beneficial amino acid composition for human consumption. Its vitamin and mineral content is comparable to other pulses, and it has a low fat content dominated by unsaturated lipids. There are no specific problems concerning toxic or allergenic substances, and no unusual anti-nutrients. However, the low levels of the crop in diets mean there is no significant benefit in terms of these nutrients at present.

A number of dissemination activities have concentrated on WP5 in India, where a range of recipes have been tested with consumers to try to develop value-added products. Of ten basic products, there was keen interest shown in nine: *dhal*, nuggets, *kandals*, stuffed *roti*, *namkeen*, *pakoras* and sprouted *chat*, supporting the view that there is a potential market for value-added products from ricebean. Further organoleptic evaluations will be carried out over the coming year.

Management activities

Routine management activities were carried out according to the workplan. The second annual workshop was held at Kaziranga, near Jorhat in Assam, India, in October 2007, and was followed by a field tour to ricebean growing areas. The event was organised by the project staff at the Assam Agricultural University, Jorhat, and was attended by other staff from there and also by the Vice Chancellor. The meeting updated project participants on the progress of the year 1 activities, and allowed adjustment to the plans for year 2 where necessary. Detailed formats for data collection were also agreed. Experimental protocols and workplans were further elaborated during a field evaluation visit in Nepal, and a meeting for the Indian partners in Bhopal, held in 2007 and 2008 respectively.

A number of relevant networks have been joined additional to those noted in year 1, and email discussions contributed to.

The project website (www.ricebean.org) was developed by CAZS-NR, and went on-line in December 2006. It is subject to continual updating. An important feature is the extensive bibliography on ricebean and related species, which includes links to the abstracts or to the papers themselves where copyright permits. This list is continually updated. Previously a blog had been set up to allow project staff to communicate, but work is on-going to incorporate a discussion area in the website to allow project staff secure exchange of a wider range of files than would be possible with a blog. The dissemination strategy was developed during the proposal stage, and has been further refined since then. End users and intermediate users have been identified.

The GVT calendar 2008, with a distribution of 1000 copies, was largely devoted to ricebean and activities under FOSRIN. Two pamphlets by CSKHPKV, a brochure by GVT and publications in local languages by Assam were also prepared which contains the package and practices of ricebean. This will be useful to disseminate the project activities and enhancing the knowledge of farmers. In addition, 53 small scale demonstrations of ricebean were laid out on farmer's fields in HP and two in Madhya Pradesh. Many other dissemination activities have also been undertaken.

1.3 If applicable, comment on the most important problems during the period including the corrective actions undertaken

There were no major problems during the period, although some of the activities were delayed as knock-on effect of the delays in year 1. Data collection in Nepal has again been affected by a number of strikes and blockades. Although not affecting project staff directly, they have made it impossible to move in some of the rural areas where data was to have been collected. There have been some similar problems in Assam state in India.

Germplasm exchange.

We had anticipated that the accession by both India and Nepal to the International Treaty on Plant Genetic Resources for Food and. Agriculture would have made the exchange of germplasm between the two countries simpler than in the past, but this has not proved to be the case. Project staff from both India and Nepal have has a number of meetings with officials in New Delhi in order to develop a means to send seed of Indian genotypes to Nepal, but this has not yet been possible. There is, however, no problem in sending seed from Nepal into India. In the meantime, Dr Yadavendra is going ahead with the Indian crosses, which will at least give us material for any future work.

Hard-seededness.

This appears to be a particular problem in ricebean, and it could be a major problem for both farmers and consumers. Our germplasm collection needs to be screened, using a relatively simple procedure. This has already been carried out at Palampur, and results are being assessed, and will be done at all study sites in Nepal on the core collection over the next season.

Section 2 – Workpackage progress of the period

2.1 Workpackage 1: Supply chain, demand and marketing

2.1.1 Objectives

The objective of this WP, which is being conducted by CAU Kiel in conjunction with the Indian and Nepalese partners, is to derive from an empirically estimated hedonic demand function a legumes trait value index for guiding ricebean breeding, and to design a strategy for introducing ricebean into the legumes supply-chains of India and Nepal.

Specific objectives for this period are to

(1) Review the scientific literature on the supply chain analysis in developing countries;

(2) consult with experts on methods for analysing the chemical composition of ricebean;

(3) design and test a battery of laboratory analyses for measuring ricebean characteristics;

(4) plan in detailed field work in India and Nepal;

(5) carry out field work in India and Nepal;

1. gathering of ricebean samples;
2. surveying agents of the ricebean supply chain.

2.1.2 Summary of work in year 1

In WP1 the literature on the estimation of the hedonic demand functions and on supply chain analysis was surveyed by CAU Kiel, and digital biographies assembled. A robust set of laboratory analyses for measuring ricebean and other pulse characteristics were designed and pre-tested, and a number of samples collected for analysis. This will eventually enable the development of the Market-price based Legumes Trait Value Index – a tool to allot a monetary value to particular traits of a variety which can be used to guide plant breeders when selecting traits to breed for. Methods for the field work were developed and presented in a conference, and linkages were established to enable the main field work to begin in 2007, in consultation with the Asian partners.

2.1.3 Progress towards objectives in year 2:

(1) Review the scientific literature on the supply chain analysis in developing countries

- the relevant literature on supply chain analysis has been reviewed;
- a working paper on the approaches to supply network analysis of the flow of pulses from producer to consumer in India and Nepal has been drafted and is approaching completion.

(2) Consult with experts on methods for analysing the chemical composition of ricebean

- Established laboratory analyses for pulses have been reviewed with the assistance of two scientists of the University at Kiel (Dr. Eva Maria Hubbermann, Institute of Human Nutrition and Food Science, Department of Food Technology; Dr. Ralf Blank, Institute of Animal Nutrition and Physiology).

(3) Design and test a battery of laboratory analyses for measuring ricebean characteristics

- A range of methods have been selected taken into account their laboratory availability in Nepal and India;
- samples of ricebean and other pulses were collected in Kathmandu (Nepal) in March 2007;
- a battery of laboratory analyses was used to measure cryptic and evident characteristics of these pulses (for details see Table 2.1.1).

(4) Plan in detail field work in India and Nepal
- Areas from where ricebean samples and market intelligence was to be collected was determined in consultation with project collaborators from India and Nepal at the FOSRIN meeting in November 2007 in Assam;
- details of the travel schedule was worked out e-mail.

1) Carry out field work in India and Nepal

a) Collection and analysis of ricebean samples
- Travelled through Nepal and India to collect samples;
- 114 samples of ricebean were collected in Nepal and 53 in India;
- samples from Nepal are analysed in Li-Bird's lab in Pokhara;
- samples from India are analysed in the lab from Anand Agricultural University;

b) Surveying agents of the ricebean supply chain
- 11 farmers and 51 traders were surveyed in Nepal
- 22 farmers and 29 traders were asked about ricebean trading in India.

Table 2.1.1: Battery of analyses for ricebean

Ricebean characteristic	Used method
1. Fat content	Ether extract
2. Protein content	Kjeldahl
3. Water content	Loss on drying at 95-100°C
4. Ash content	Loss by burning in a furnace at 550°C
5. Crude fibre content	Loss of weight after digestion
6. Carbohydrates content	Calculation by difference
7. 100-seed weight	Weight of 100 seeds
8. Swelling capacity	Weight before and after soaking
9. Water up-take	Volume before and after soaking
10. Size and form	Length/ breadth ratio
11. Colour	Share of different colours in one sample
12. Share foreign matter	Weight of foreign matter

Results

Nepal

Table 2.1.2: Key characteristics of 11 farmers surveyed in Nepal, first quarter 2008

	Ricebean					
	Area in m²	Yield in kg	Sold amount kg	% Home-consumption	Selling price NPR*/ kg	Selling price €**/kg
Mean	439	75	54	36	27.75	0.304
Median	225	72	24	20	27.25	0.298

*NPR: Nepalese Rupees

**€: Euro

Table 2.1.2 summarizes characteristics of ricebean growers who we have surveyed in Nepal. The variable "Area" in the first column stands for the estimated average area on which farmers grow ricebean. "Yield" is shown per farmer and not per area because farmers do not measure and record ricebean yields per unit area. The variable "Sold amount" represents the quantity of ricebean sold on the market. "Home consumption" denotes the proportional amount that was consumed in each household. The last two columns give March 2008 prices of ricebean in Nepalese Rupees and in Euro. For all conversions of Nepalese and Indian Rupees into Euro exchange rates from the Association of German Banks on the 14th of February were used.

There are significant differences between the areas farmers are using to grow ricebeans. The smallest ricebean field of a farmer was 20 m² and the largest was 1500 m². Reportedly, factors influencing the size of ricebean area were land inclination, access to irrigation, and farmers' cropping systems. The large ricebean fields were found in the flat unirrigated regions of Makwanpur, Terai, Nepal. There farmer grow ricebean to exploit residual soil moisture after the main crop has been harvested.

Table 2.1.3: Mean buying and selling prices of 51 traders surveyed in Nepal, 1st quarter 2008

		Buying price		Selling price		Margin	
		NPR*/kg	€**/kg	NPR/kg	€/kg	NPR/kg	in %
Mean	Wholesaler	34.38	0.38	38.18	0.42	3.79	12
	Retailer	38.34	0.42	44.56	0.49	5.91	15

*NPR: Nepalese Rupees

**€: Euro

Table 2.1.3 shows buying and selling prices of ricebean together with retailers' and wholesalers' margins. To allow a comparison of prices in Nepal and India (Tables 2.1.3, 2.1.5) prices were converted into Euro (€).

The first two columns show ricebean purchase prices for wholesalers and retailers. The third and fourth column contains selling prices of wholesalers and retailers. The last two columns present ricebean margins, calculated as the differences between buying and selling prices. Retailers' margin is at 15 % on average slightly higher than wholesalers' margin which amounts to 12% on average. The difference does not necessarily translate into a higher profit for retailers as the trading cost of retailers sometimes includes transport costs for the beans.

The large difference between the mean selling price of farmers and mean buying price of wholesalers is most likely due to statistical averaging over price from different regions.

India

Table 2.1.4 contains the same information for ricebean farmers in India as Table 2.1.2 for farmers in Nepal. Indian farmers are selling less of their harvested ricebean on markets which implies a higher proportion (76%) of home consumption. In our sample from India, most ricebean farmers who have been surveyed were tribals.

Table 2.1.4: Key characteristics of 11 farmers surveyed in India, first quarter 2008

	Ricebean					
	Area in m²	Yield in kg	Sold amount kg	% Home-consumption	Selling price Rs*/kg	Selling price €**/kg
Mean	1167	65	38	76	17.78	0.307
Median	100	29	0	100	15.00	0.259

*Rs: Indiean Rupees

**€: Euro

Table 2.1.5 shows mean buying and selling prices of Indian retailers and wholesalers together with their margins. As in Nepal retailers have higher prices than wholesaler but this time both stages are having the same margin.

Table 2.1.5: Mean buying and selling prices of 29 traders surveyed in India, 1st quarter 2008

		Buying price		Selling price		Margin	
		Rs*/kg	€**/kg	Rs/kg	€/kg	Rs/kg	in %
Mean	Wholesaler	16.80	0.29	19.83	0.34	3.03	19
	Retailer	20.12	0.35	23.89	0.41	3.78	19

*Rs: Indian Rupees

**€: Euro

General observations on ricebean supply chains

- ricebean is mainly grown by farmers in remote areas far from markets and where transport costs for farmers are high;
- ricebean supply chains tend to involve only a small number of stages and in some areas there is only on stage separating producers from consumers;
- supply chains in Nepal tend to be longer than in India; in Nepal they connect farmers with retailers in major cities whereas in India ricebean often does not reach consumers in major cities;
- ricebean may lack marketability because of its heterogeneous appearance in size and colour.

A preliminary evaluation of the grain quality of various legumes (physical parameters, cooking and soaking qualities and nutritional composition analysis) was carried out in the Food Research Laboratory of NARC at Khumaltar. This information was generated to support the research on supply chain characterisation in ricebean, and was used to compare the nutritional composition of ricebean with legumes that are more often found in the diet in Nepal (Table 2.1.6 and Table 2.1.7). In Nepal, mung bean is regarded as a nutritious legume with high bioavailability and is generally recommended for use by sick people. The nutritional data are similar to mung bean for all parameters analysed, rejecting the widely held perception and beliefs of rural people that eating ricebean disturbs the digestion.

Table 2.1.6: Nutritional composition of grains of different legumes assessed in Nepal

Legume	**Moisture (%)**	**Ash (%)**	**Protein (%)**	**Crude fat (%)**	**Crude fibre (%)**	**Carbohydrate (%)**	**Energy (Kcal/1000g)**
Ricebean	13.8	4.5	24.0	0.8	4.5	52.4	312.8
Kidney bean	15.3	4.1	22.9	2.2	3.9	51.6	317.7
Peas	12.8	3.1	23.8	1.5	5.4	53.3	322.7
Mung bean	12.5	4.1	24.7	1.4	4.6	52.6	321.8
Chick pea	12.6	3.1	18.7	6.3	5.7	53.6	345.5
Cowpea	14.6	3.5	26.8	2.0	4.1	49.1	321.1
Black gram	13.1	3.4	25.3	2.1	4.7	51.4	325.7
Horsegram	12.8	4.3	23.4	0.9	4.5	54.1	318.1
Soybean	11.0	5.4	39.2	19.3	5.0	20.1	411.1

Table 2.1.7: Physical characteristics, soaking and cooking behaviour of kernels of different legumes assessed in Nepal

Legume	Length (mm)	Width (mm)	L/W ratio	1000 grains (g)	Bulk density (g/l)	Water absorption after soaking (%)	Water absorption after cooking (%)
Ricebean	8.0	5.1	1.6	108	856	175	103
Kidney bean	14.9	7.4	2.0	452	755	98	107
Peas	7.3	6.6	1.1	225	814	100	106
Mung bean	4.8	3.7	1.3	43	829	130	132
Chick pea	9.9	7.2	1.4	287	791	114	117
Cowpea	7.6	5.8	1.3	NA	812	NA	NA
Black gram	5.0	3.9	1.3	39	866	126	157
Horsegram	5.7	4.0	1.4	29	854	101	104
Soybean	8.2	7.2	1.1	200	740	122	120

LI-BIRD assisted in sample collection and market survey of ricebean in different market potential areas of Nepal. This involved selection of potential areas of ricebean cultivation, sample collection along with market information and in market survey in January to March 2008.

2.2 Workpackage 2: Genetic diversity and indigenous knowledge

2.2.1 Objectives

WP2, led by LI-BIRD (Partner 8), with additional inputs from the other Asian partners, involves the assessment of genetic diversity and indigenous knowledge on ricebean. It has the following four objectives:

- To describe the extent of ricebean diversity and its geographical distribution
- To collect a representative sample of ricebean germplasm together with associated socio-economic data
- To evaluate the collections in the field for phenotypic diversity analysis and,
- To understand the socio-economic and bio-physical factors controlling ricebean diversity and its utilization

A number of activities such as collection of representative samples of germplasm from across the country with the associated socio-economic information and on-farm evaluation of the collections over the seasons and in different cropping systems have already been started. Analysis, evaluation and identification of promising accessions are on-going. Farmers' local knowledge associated with the crop from ricebean growing areas was documented and analysed. Table 2.2.1 summarises the objectives, the respective work performed and major deliverables.

Table 2.2.1. Summary of objectives and work performed during reporting period

Objectives	Worked performed	Deliverables
2.1 To describe the extent of ricebean diversity and its geographical distribution	Assessment of potential ricebean growing districts in Nepal. Area under ricebean in each district assessed and districts were categorized on the basis of area coverage	Report of distribution of ricebean in India and Nepal prepared and submitted in May 2007
2.2 To collect a representative sample of ricebean germplasm together with associated socio-economic data	Along with 156 accessions of ricebean landraces in 2006, additional accessions from Kaski and Ramechhap were collected in 2007. 33 ricebean accessions from home gardens of Chitwan valley were also collected in 2007/08.	Data from two years trials and seed samples available for evaluation Agro morphological data of accessions from Chitwan valley, and seed samples, available
2.3 To evaluate the collections in the field for phenotypic diversity analysis	50 core and 66 non-core accessions evaluated on-farm under intercropping system and sole cropping system respectively in Gulmi.	Promising landraces identified for further evaluation and promotion
2.4 To understand the socio-economic and bio-physical factors controlling ricebean diversity and its utilization	Farmers' local knowledge associated with production, diversity and utilization of ricebean from potential ricebean areas documented and analysed. Reconnaissance survey conducted in Chitwan valley to assess the traits of ricebean grown in home gardens and preferred by farmers	Deliverable on farmers' indigenous knowledge produced in March 2008 Findings of preferred traits and agro morphological features already compiled

2.2.2 Summary of work in year 1

An initial assessment of potential ricebean-growing districts was carried out in Nepal. In addition, over 150 ricebean accessions were collected from 16 districts in Nepal, together with over 100 from the historical collection at NARC and almost 90 from 16 districts in India. Local knowledge on the crop was also collected in both countries. The collected accessions from Nepal were evaluated for agro-morphological traits and phenotypic diversity on-farm at Gulmi in the middle hills by LI-BIRD, and at NARC headquarters. The data collected showed that ricebean could be grown in a range of climatic conditions, but in Nepal was mostly found in drought-prone sloping areas as well as on unirrigated flat river fans between 700 and 1300 m. It is also grown in home gardens from 200 m in Chitwan up to 2000 m in Ilam district. Passport data was recorded using standard procedures developed by the project partners.

The germplasm evaluation was carried out under normal farmers' conditions without chemical fertiliser, although spraying was carried out against pests – standard protocols were developed and used to record the data. The on-farm evaluation showed considerable variation between accessions in time to maturity, seed size and colour, time to maturity and yield. The NARC evaluation also showed differences in growth habit. Principal Components Analysis showed that a cluster of genotypes from the mid to high hills in W Nepal were similar. The degree of diversity shown suggested considerable possibility for plant breeding to improve the crop.

Assessment of diversity as seen by farmers identified four main types of ricebean in each of Gulmi and Ilam districts in Nepal, and seven in India (although in Himachal Pradesh only a mixture of landraces was grown). The main criteria used by farmers were grain size and colour, maturity, and growth habit. In both countries, ricebean was grown mainly as an intercrop or a mixed crop with maize, as well as on its own as a sole crop, although in some areas it was mixed with cowpea or sorghum. Most of the crop was planted in June before the onset of the monsoon. Farmers identified a number of production constraints including low yield potential, availability of other legumes in the market, unavailability of improved material, and low interest by research and extension services. They were aware of a number of medicinal and nutritional benefits, and noted that ricebean had cultural significance in a number of areas. However, they were also aware of its flatulence-causing properties. There were no established markets for ricebean – it was not grown for commercial purposes and was usually exchanged between farmers or sold in local markets.

2.2.3. Activities of Work Package 2

Field visit to ricebean districts

From an initial assessment of the area coverage of ricebean using the expert knowledge of the District Agriculture Development Offices (DADOs) in 2006, districts were categorised as high, medium and low area coverage (Figure 2.2.1). Districts with higher area coverage, Tanahu, Baglung, Ramechhap, Sindhuli, Dolakha and Gulmi, were visited to further validate this information regarding ricebean distribution, diversity and cultivation system in 2007. DADO offices were visited first to identify the most important Village Development Committee (VDCs) and with them to identify the important areas for ricebean cultivation. A joint field visit with DADO staff to these areas was also arranged. A report on national distribution of ricebean has already been submitted as Deliverable 2.1. Table 2.2.2 shows the ricebean areas and major diversity of ricebean in districts where it is grown.

On-farm trial and evaluation

In April 2007, we participated in a national planning meeting held in Kathmandu and coordinated by CAZS-NR. Various field activities and their trial protocols were discussed in detail and agreed (Annex 2). LI-BIRD rented about 2000m^2 (4 *Ropani*) of *bari* land from two farmers, Top Bahadur Thapa and Jit Bahadur Thapa, in Bajarmare, Darbar-Devisthan-5, Gulmi, in Nepal in June 2007 (Figure 2.2.2).

Table 2.2.2. Important ricebean growing areas and ricebean diversity in Nepal

District	Small areas/VDCs	Major diversity	Local name
Tanahun	Ambu, Chhimkeshowri, Deurali, Dharampani, Chhipchhipe, Baidi, Kota, Virkot, Gajkot, Kinhu, Raipur, Firfire	*Kalo sano, seto sano, Chhirkemirke thulo, Chhirkemirke sano*	*Masyang*
Baglung	Paiyu Thunthap, Rangkhani, Sarkuwa, Jaidi, Chhisti, Narayansthan, Tityang, Siyana, Bhakunde, Resha, Biyu, Hatiya, Harichaur	*Chhirkemirke (sano), sano seto , Kalo sano, Khairo thulo, Rato sano*	*Siltung/Saltung/ Ratamas*
Gulmi	Darar Devisthan, Simichaur, Dubichaur, Birbas, Gaudakot, Hardineta, Baletaxar, Amararbathok, Kharjyang, Digam, Ruru	*Bhadure, Rato, Seto, Chhirkemirke, Thulo pinyalo*	*Jhilinge*
Ramechhap	Sukajor, Okhreni, Sunarpani, Himganga, Rampur, Ramechhap, Bhaluajor, Pakarbas, Makathum	*Chhirkemirke thulo (Bage), Rato, Seto sano, Kalo, Pahelo*	*Masyang*
Sindhuli	Bitijor, Bhuwaneshwori, Tinkanya, Ranichuri, Kapilakot, Dadigurase, Kamalamai Municipality	*Chhirkemirke, Seto, Khairo, Kalo*	*Masyang*
Dolakha	Sahare, Melung, Jafe, Chyama, Dadakharka, Bhedpu, Bhirkot	*Chhirkemirke, Seto, Khairo, Rato*	*Masyang*

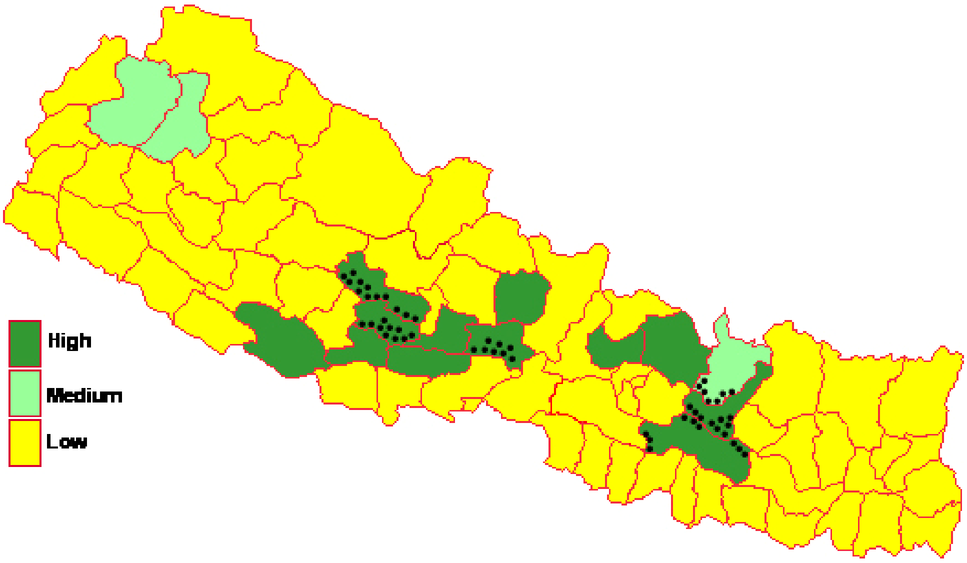


Figure 2.2.1. The main ricebean growing districts in Nepal. The information is based on expert knowledge. High, medium and low indicates the order of the area coverage, and dots represent pocket areas for ricebean within the main ricebean-growing districts.

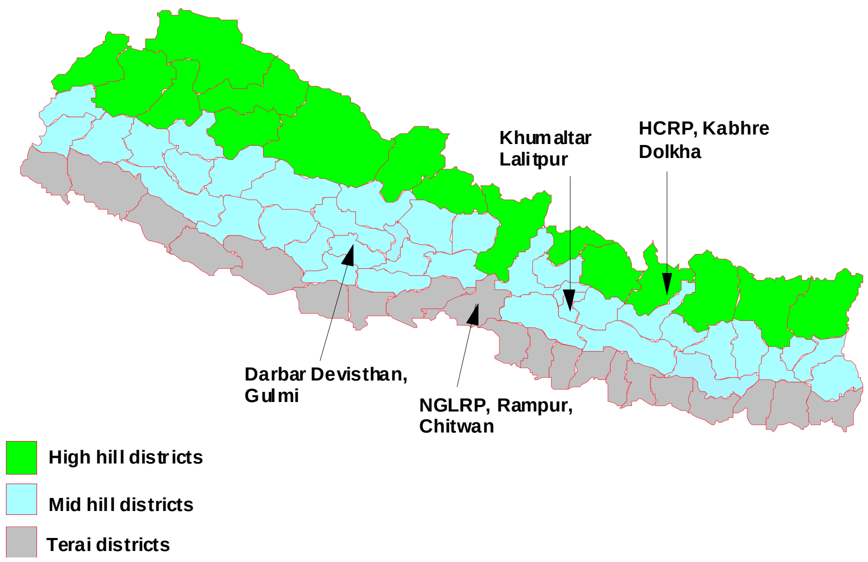


Figure 2.2.2. Ricebean testing sites across three agro-ecological regions in Nepal

Agro-morphological characterization in field

A total of 218 ricebean accessions were evaluated in field plots in four growing environments from the lowlands to the mid-hills of Nepal (Annex 1; Figure 2.2.2). These were collected during various collaborative germplasm exploration missions from 1972 to 1994, and in 2006. The germplasm was obtained mainly from household stores, and was stored in the gene bank in ABD, NARC, Khumaltar, with some also maintained by LIBIRD.

During 2007, the collection was grown in the field for evaluation and agro-morphological characterisation, classified as a core and a non-core collection (Annexe 3, Tables A3-1 – A3-4). Details of field plots, methodology and observations were according to the protocol developed by the project team (Annex 2). The plots were managed using the usual farmers' practices, so neither fertilizer nor irrigation were applied. Across the sites, 25-28 agro-morphological traits, including seed characteristics, were recorded at different growth stages according to standard methods[34] (Annex 2, Annexe 4). These descriptors include phenology, yield and yield components, other quantitative and qualitative traits. In addition, disease and pest reactions were recorded. Quantitative and qualitative traits were recorded on 5 individual plants per plot, while phenological observations were based on total plant population per plot. Results from the core and non-core accessions are presented and explained separately.

Core collections under intercropping with maize

A set of 50 diverse accessions from the collection made by NARC and LI-BIRD in 2006 were categorized as core accessions (Annex 3, Table A3-1). These were planted as a maize intercrop in Gulmi, in 3.75 x 2 m plots with two replications (See Figure A2_1 in Annexe 2). Five maize rows were planted using the standard maize spacing of 75 x 25 cm during the last week of April, and ricebean was planted between maize rows in the first week of June. There were 40 maize and 16 ricebean plants per plot. Agro-morphological and agronomic traits of the core and non-core accessions were recorded using agreed descriptors (Annexe 5).

Non core collections under sole cropping

A total of 66 accessions collected in 2006 and 2007 were planted as a non-core collection in Gulmi (Annex 3, Table A3-4). This was planted as a sole crop using a plot size of 2 x 2.5 m per accession (similar to Figure 2 in Annexe 2, with modified dimensions). Staking was provided for those landraces with indeterminate growth habit.

[34] Anonymous, 1995. Descriptors for grain legumes and oil seed crops. The Plant Genetic Resources Centre, Department of Agriculture, Gannoruwa, Peradeniya, Sri Lanka.

Field staff regularly supervised and monitored the trials, and project staff visited at planting, during active growth, and at flowering and harvesting. Data on various agro-morphological traits of ricebean were collected according to the protocol (Annexe 2). Accessions from core and non-core collection were characterised at different growth stages. Five plants per plot were randomly selected for characterization. However, accessions with low germination data were collected from less than 5 plants. The format for data collection for characterization is attached in Annex 4. Agro morphological and agronomic trait data were recorded on the basis of agreed descriptors (Annex 4). During the second week of July, project partners monitored the on-farm activities in Gulmi, including the trials for core and non-core accessions and mother trials conducted in Darbar Devisthan and Simichaur VDC.

Germplasm collection in India

In India, exploratory visits (Table 2.2.3) were made during 2007 in ricebean growing areas of Madhya Pradesh, Assam, Mizoram, Orissa, Himachal Pradesh and Uttarakhand by Indian partners in their respective working areas for the collection of germplasm, which was done by visiting individual households from their stores as the crop was not in the field during exploration. This work had been scheduled for 2006/07, but was delayed.

Table 2.2.3: Area surveyed and germplasm collected during 2007-08, additional to 2006/07

Location	No.of accessions	Name of partner
Chhattisgadh, Orissa and Uttarakhand	16+44*	GVT
Mandi, Solan, Bilaspur and Kangra, Himachal Pradesh; Uttarakhand	78+ 21*	CSKHPAU
Karbianglong, Assam; Aizwal and Mamit, Mizoram; and Ganjam, Koraput, Phulbani, Rayagada, and Nabarangpur; Orissa	57	AAU

* Collected during March 2008 and will be evaluated during 2008

The germplasm samples were evaluated during *kharif* 2007 (rainy season) in farmers' fields in Bhagore village by GVT, and on university experimental farms by CSKHPAU and Assam. Each germplasm line was grown in a single row with 45 cm row spacing. The different phenological characters and yield data were recorded (Annexe 4) in order to assess variation:

Results

Core and non-core collections, Nepal:

In Gulmi, the summary of variation for accessions for selected traits in the core and non-core collections is presented in Tables 2.2.4 and 2.2.5, while additional traits are shown in Annex 7. One of the replications of each of the core and non-core collections was badly damaged by rabbits, and this resulted in complete loss of data for some of the accessions and very erratic data for others. Those data were excluded from the analysis.

Although, it is difficult to draw much inference from data from a single replicate, the general trend for variation is remarkable. For example, plant height of the core accessions ranged from 14 – 450 cm, days to flowering 105 – 130, days to maturity from 107 – 180, and 100 seed weight from 6 to 24 gram. The most variable traits were plant height, days to maturity, number of pods per plant and hundred seed weight (Tables 2.2.4 and 2.2.5). Data for both core and non-core collections agree in general for the traits described above, except for hundred seed weight which showed great variation in the core collection but not in the non-core collection.

Table 2.2.4. Descriptive analysis of quantitative traits of 50 ricebean core collections evaluated in Gulmi, Nepal 2007

Quantitative traits	Mean	Range	Standard deviation
Plant height	149	14-450	86.11
Days to flowering	114	105-131	11.22
Days to maturity	162	107-180	14.08
Pod length (cm)	9	6-13.3	1.6
Number of pods per plant	65	17-192	50.7
Number of seeds per pod	7.8	5-9	1.55
Hundred grain weight (g)	9.9	6-24	18.24

Table 2.2.5. Descriptive analysis of quantitative traits of ricebean non-core collections evaluated in Gulmi, Nepal 2007

Quantitative traits	Mean	Range	Standard deviation
Plant height	50.9	15-233	40.72
Days to flowering	117	104-130	9.04
Days to maturity	164	86-	20.94
Pod length (cm)	8	4.4-13.5	1.71
Number of pods per plant	41	5-170	51.0
Number of seeds per pod	7.3	2.3-10	1.96
Hundred grain weight	9	6-14	1.64

At CSKHPKV, Palampur, the experiment was initially assessed as an augmented design, and analysis was carried out for seed yield and other related traits. Data (not presented) showed significant differences between genotypes for days to 50% flowering, flowering period, days to first mature pod, terminal leaflet blade width, pod length, 100- seed weight, dry biomass per plant, and seed yield per plant. Partitioning between check lines and accessions showed significant differences for all traits except days to first mature pod, terminal leaflet blade length, pods per plant and seed yield per plant.

Based on these data and on observation of the standing crop, promising accessions will be identified for observation nursery and mother trials in June 2008.

2.3.3 Local knowledge documentation and analysis

The local knowledge associated with ricebean in Nepal was assessed using a range of activities and social tools: direct field visits to ricebean growers, focus group discussions and semi structured interviews from different pocket areas. A report on local knowledge documentation and analysis (Deliverable 2.2) was produced in March 2008 and is undergoing final edits: it is annexed to this Report.

The area under ricebean in each district of Nepal was earlier assessed by the project team while investigating the national distribution of the crop. On the basis of information gathered during prior visits and on site accessibility, Gulmi and Ramechhap were selected from within the important districts for an in-depth documentation and analysis of local knowledge. Pocket areas within those two districts were identified in collaboration with District Agricultural Development Offices (DADOs). Darbar Devisthan Village Development Committee (VDC) in Gulmi and three VDCs; Ramechhap, Bhaluajor and Pakarbas, in Ramechhap districts, were identified for this work.

A desk review was carried out to identify appropriate methodologies for the documentation of knowledge associated with ricebean, using the following steps:

- Review of literature on local knowledge for various practices from published and unpublished documents, proceedings, papers, reports.
- Review of various procedures adapted in the past by other LI-BIRD projects while documenting local knowledge.

- Interaction with professionals within organization for seeking suggestions.
- Internet based web search

A draft methodology was finalized in consultation with the professional staff of LI-BIRD. A four stage strategy of Knowledge Based System (KBS) approach, applied in the past for agro-forestry research and extension[35], was regarded as appropriate to document the local knowledge associated with ricebean.

The project team identified research questions and a checklist to document the local knowledge associated with ricebean. The research questions are presented in Annex: 8. Then, Focus Group Discussions (FGD) were conducted in the selected study areas by interviewing knowledgeable persons. Altitude range, gender and ethnicity were considered while selecting the key informants. In Darbar Devisthan, 15 key informants were identified, while there were 18 informants from the three VDCs in Ramechhap.

Farmers' local knowledge associated with production, diversity and utilization of ricebean was documented from the selected study areas. A Master's level student from Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan documented the farmers' indigenous knowledge on ricebean in Ramechhap as a part of his research. Documented knowledge from Gulmi and Ramechhap was then compiled and a report produced in March 2008.

2.3.4 Reconnaissance survey to assess the preferred traits of ricebean in home gardens in Chitwan valley

Farmers also grow ricebean as a vegetable in their home gardens, particularly in the plain areas of Nepal, and a reconnaissance survey was conducted in the Chitwan valley to investigate this. Altogether 33 growers were purposively asked for information, and project staff visited and observed home gardens and collected seed samples. Ricebean pods are used for green vegetable, dried whole beans are boiled to make soup, primed whole grains are fried to make snacks (*biramla*), and split grains are used to make soup (*dal*).

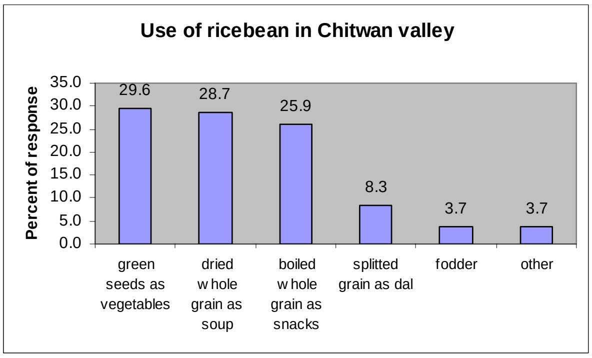


Figure 2.2.5. Different use of ricebean with percentage of responses in Chitwan

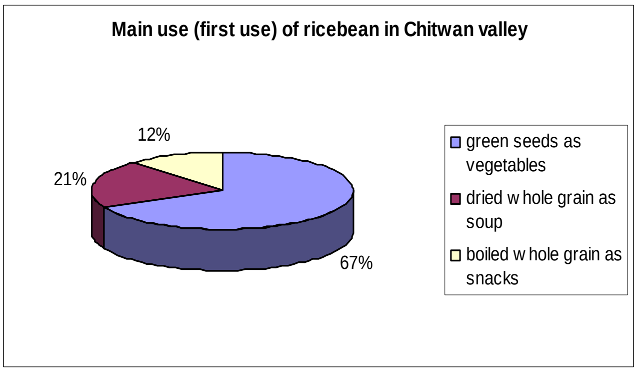


Figure 2.2.6. First use of ricebean with percentage of respondents in Chitwan

Of the total of 108 responses, 29.6% have used the green pods as vegetables. The second most popular use was the dried whole grain as *dal* (28.7%), and boiled whole grain as a snack (*biramla*): fewer farmers used split grain as *dal* or for livestock fodder (Figure 2.2.5). Similarly, while asking about the first use of ricebean with 34 respondents, using the green pods as a vegetable was the first use of ricebean (67% of respondents) (Figure 2.2.6). This indicates that although ricebean has multiple uses, growing it as a green vegetable is the most common in home gardens of Chitwan Valley.

[35] Walker, DH, Sinclair, FL & Thapa, B (1995) Incorporation of indigenous knowledge and perspectives in agroforestry development. Part 1: review of methods and their application. Agrofor Sys **30**: 235-248

Table 2.2.6. Use of ricebean and preferred traits by farmers in home gardens in Chitwan, 2007

Utility	Preferred traits	Respondents (%)
Green vegetable	Indeterminate growth habit which allows multiple harvesting	23.5
	Long pods	47.1
	Bold seeds	70.6
	High yield with more pods per plant	32.4
Boiled grain as snacks (*Biramla*)	Bold seeds	52.9
	High yielding	11.7
	Less time for cooking	5.8
	Long pods	5.8
	Light coloured grains	11.7
	Dark coloured grains	11.7
Whole grain soup	Bold seeds	53.8
	Light coloured grains	15.4
	Less time for cooking	23.1

Whatever the usage, farmers prefer bold seeded varieties with high yield. However, the preferences for other uses vary. For example, long pods with indeterminate growth habit are preferred for use as a green vegetable. For *dal* and *biramla*, light coloured grains that require relatively less time for cooking are preferred (Table 2.2.6).

Seed samples of 33 different accessions were collected from the home gardens in Chitwan and their agromorphological features noted. There was a wide range of variation in seed size and other traits (Table 2.2.7).

Table 2.2.7: Diversity in qualitative traits of ricebean grown in home gardens in Chitwan, 2007

Trait	Category of triats	Number of accessions
Leaflet shape	Lobed	10
	Deltoid	19
	Ovate	3
	Cuneate	1
Leaf pubescence	Sparsely pubescent	22
	Moderately pubescent	11
Petiole colour	Light green	22
	Green	10
	Greenish purple	1
Leaf colour	Light green	2
	Intermediate green	22
	Dark green	9
Stem colour	Light green	24
	Green	2
	Light green with purple patches	7
Colour of upper suture of immature pod	Blackish	30
	Green	1
	Light green	2
Mature pod colour	Brown	16
	Dark brown	14
	Reddish brown	3
Seed colour	Light yellow	6
	Mottled	21
	Black	5
	Red type	1

Table 2.2.8. Descriptive analysis of quantitative traits of 33 ricebean landraces grown in home gardens in Chitwan

Quantitative traits	Mean	Range	Standard deviation	Coefficient of variation
Leaflet length	11.1	7.1-13.4	1.376	12.35
Leaflet width	8.2	4.8-10.46	1.263	15.44
Peduncle length	10.8	6.7-13.1	1.610	14.95
Number of seeds per pod	8.1	6.8-9.4	0.623	7.70
Number of pods per peduncle	4.3	3.0-6.6	0.851	19.60
Thousand grain weight	145.9	39-255.6	58.8	40.28

A descriptive analysis of the quantitative traits for accessions grown in Chitwan valley is shown in Table 2.2.8. The thousand grain weight ranged between 39- 255.6 g and this clearly showed that the ricebean population varied in grain size. Variation for thousand grain weight was very high as also indicated by the high standard deviation (58.8%).

2.3 Workpackage 3: Molecular markers

2.3.1 Objectives

WP3 addresses the characterization of ricebean diversity using molecular markers, and is led by NARC, with assistance from CAZS-NR.

***Table 2.3.1*:** Summary of the objectives and activities deliverables for WP3

Objectives	Activities performed	Deliverables
To evaluate and characterize the ricebean germplasm	Agro-morphological characterisation and evaluation of ricebean landraces: • On-farm/on-station evaluation of accessions in core collections (50 accessions) • On-farm/station evaluation in non-core collections (168 accessions)	218 ricebean accessions characterised and key traits and best performed accessions identified for further evaluation
To develop the protocol	Stratified sampling of the germplasm	A set of 38 stratified sample of ricebean germplasm identified
	Isolation of DNAs	Individual DNA from stratified samples of ricebean isolated
	Optimisation of the protocol	18 cowpea SSR markers were tested in different concentrations and thermal cycling conditions
To identify a set of polymorphic SSR markers	Screening of SSR polymorphic markers	109 adzuki bean SSR markers and 18 *Vigna* (cowpea) SSRs analysed on the set of stratified sample of ricebean accessions identified from the preliminary agro-morphological evaluation.
	Identification of polymorphic SSR markers	13 adzuki bean SSRs found with medium to high PIC content useful for detection of molecular diversity in ricebean germplasm

The National Grain Legume Research Programme (NGLRP) is one of NARC's commodity programmes, and undertakes research to improve legume crops. Unfortunately, ricebean is not included in the national legume programme as it is considered to be a minor crop and therefore no systematic studies on its potential, diversity and other attributes have been initiated in Nepal. A collection of ricebean germplasm held by the NARC genebank from 29 districts, and a collection of accessions collected in this project by LIBIRD from 16 districts in 2006, were studied for agro-morphological characterisation and evaluation in diverse growing environments in the 2007 season[36].

Farmers classify these landraces according to days to maturity, seed colour and grain size, but no systematic study or breeding has been carried to give greater understanding of ricebean diversity and genetic structure. However, the germplasm is thought to possess valuable diversity and a preliminary evaluation in the first year of the project confirmed this. These variations may be useful for breeding improved varieties with desired traits. In WP3, agro-

[36] ABD, 2004. Annual report of Agriculture Botany Division (ABD), NARC. Khumaltar, Lalitpur, Nepal: ABD

morphological characterization and evaluation of local germplasm, and an SSR diversity analysis, were carried out in the field and laboratory in 2007 in order to determine the agro-morphological diversity of the germplasm, to analyze the molecular marker data using SSRs, and to identify polymorphic *loci* (markers). The primers for these *loci* will then be used to investigate the molecular diversity in local ricebean germplasm. The relevant project objective is "to characterise (ricebean) diversity using molecular marker techniques", and tasks for the current period are "to extract and assay DNA for hypervariable markers in bulk and individual plant samples of selected accessions", and "to assay for polymorphic markers."

2.3.2. Work in year 1

During the first year, a stratified sample of 34 germplasm accessions collected in WP2, based upon agro-ecological diversity, diversity of local names, and seed availability was selected. DNA was isolated from these using standard protocols to try to screen SSR markers from other *Vigna* spp. and detect polymorphic markers for the many Nepalese genotypes to be screened. This was not successful in amplifying the DNA, and the work continued in year 2.

2.3.3 Progress towards objectives

Activities

Both molecular and morphological analysis was carried out in 2007. A core collection of 50 accessions was screened at four sites, as well as a non-core collection, and both quantitative and qualitative traits were assessed. Diversity was assessed using the Shannon Weaver diversity index.

For the molecular work, 27 accessions from the NPGR/LRGR collections together with two checks (bold grained ricebean and adzuki bean were screened. Accessions were selected based on determinate or indeterminate type, seed availability, colour diversity, diversity of local name, and collection site. The adzuki bean SSR markers worked with the ricebean DNAs, with good amplification. Seventy-two SSRs of different linkage groups were tested, and 46 (74%) were amplified with 1 – 4 alleles. Overall, 28 (61%) were found to be polymorphic with two – four alleles. As a result, optimum PCR conditions and thermal cycling with an annealing temperature of 50° for ricebean PCR were determined and the adzuki bean primers optimised. A set of polymorphic loci were determined, and markers with high PIC values identified for use in diversity analysis of comprehensive ricebean collections.

This will allow the next stage of the work to begin – identification of the comprehensive collection of accessions for molecular diversity analysis, and the comparison of this diversity with phonotypic, biophysical and socio-cultural variables.

Agro-morphological characterization in field

This section summarises the results from the on-station studies done by NARC in a sole cropping system. The trials were held at the NGLRP, Khumaltar, near Kathmandu, at the Agricultural Botany Division (ABD) of NARC also at Khumaltar, and at the HC Reseacrh Programme near Khabre. Other work was carried out as part of WP2 and is reported there. The observed variations in agro-morphological traits were compared between genotypes and across the study sites, using basic descriptive diversity analysis and univariate analysis.

Screening of primers for polymorphic loci

SSRs for adzuki beans were used to identify polymorphic loci to be used for genetic distinctness and detecting marker diversity in ricebean. For this, a stratified set of 27 ricebean genotypes was identified based on agro-ecological diversity, diversity in local names, growth habit and grain traits, as well as on the quantity of seed available (Table 2.3.2). The set also included two checks, bold grained ricebean, and adzuki bean, which were used to optimise the protocol.

Table 2.3.2 Stratified accessions of ricebean used to screen SSR primers for identification of polymorphic primers.

Accessions	District of collection	Ecological region	Year collected	Remarks
Rice bean (bold)	Illam	Check sample	2006	Collected by Dr P. Anderson
Adzuki bean	Bangor	Check sample		Received from Dr P. A. Hollington, UK commercial sample
NPGR 00007	Nuwakot	Mid-hill, Central Nepal	1987	indeterminate, greenish yellow, cylindrical seeds
NPGR 00010	Lalitpur	Mid-hill, Central Nepal	1987	indeterminate, greenish yellow, cylindrical seeds
NPGR 00015	Bhaktapur	Mid-hill, Central Nepal		indeterminate, greenish yellow, cylindrical seeds
NPGR 00073	Gulmi	Mid-hill, Western Nepal	1988	indeterminate, mottled, drum shaped seeds
NPGR 00076	Arghakhanchi	High-hill, Western Nepal	1976	indeterminate, mottled, drum shaped seeds
NPGR 00087	Pyuthan	High-hill, Western Nepal	1993	indeterminate, greenish yellow, drum shaped seeds
NPGR 00090	Dang	Terai, Western Nepal		indeterminate, mottled , cylindrical seeds
NPGR 00194	Kavre	Mid-hill, Central Nepal	1987	indeterminate, greenish yellow, drum shaped seeds
NPGR 01975	Baitadi	Mid-hill, Western Nepal	1985	indeterminate, greenish yellow, cylindrical seeds
NPGR 05364	Bhojpur	High-hill, Eastern Nepal		indeterminate, greenish yellow, cylindrical seeds
NPGR 05370	Terhathum	High-hill, Eastern Nepal	1997	medium, red, cylindrical seeds
NPGR 05373	Gorkha	High-hill, Western Nepal	1987	indeterminate, greenish yellow, cylindrical seeds
NPGR 05377	Lamjung	High-hill, Western Nepal	1987	indeterminate, black, cylindrical seeds
NPGR 05382	Tanahu	Mid-hill, Western Nepal	1994	indeterminate, greenish yellow, cylindrical seeds
NPGR 05384	Mugu	High-hill, Western Nepal	1987	determinate, greenish yellow, globose seed
NPGR 06756	Humla	High-hill, Western Nepal	1988	determinate, greenish yellow, cylindrical seed
NPGR 05391	Bajura	Mid-hill, Western Nepal	1982	medium, greenish yellow, cylindrical seed
NPGR 05396	Illam	Mid-hill, Eastern Nepal	1997	indeterminate, greenish yellow, drum shaped seeds
NPGR 05420	Dhankuta	Mid-hill, Eastern Nepal	1987	indeterminate, red drum shaped seeds
NPGR 08380	Myagdi	Mid-hill, Western Nepal	1987	medium, mottled drum shaped
NPGR 07882	Bajhyang	High-hill, Western Nepal	1976	medium, red cylindrical seeds
NPGR 09391	Syangja	Mid-hill, Western Nepal	1994	indeterminate, mottled cylindrical seeds
NPGR 09461	Panchthar	High-hill, Eastern Nepal	1991	determinate, greenish yellow drum-shaped seeds
NPGR 09464	Taplejung	High-hill, Eastern Nepal	1994	indeterminate, greenish yellow cylindrical seeds
LRGR 042	Surkhet	Mid-hill, Western Nepal	2006	indeterminate, mottled cylindrical seeds
LRGR 025	Bajura	Mid-hill, Western Nepal	2006	determinate, light green, drum shaped seeds
LRGR 054	Baitadi	Mid-hill, Western Nepal	2006	determinate, greenish yellow, globose seed

Bulk DNA of 5 plants of each genotype were isolated following the modified CTAB (Cetyltrimethyl ammonium based) DNA isolation method[37]. This was done to screen SSR markers for other *Vigna* species and to detect polymorphic markers to use with a larger number of samples from Nepal and India, but unfortunately germplasm exchange between Nepal and India was not possible due to the strict regulatory requirements. The extracts were checked for DNA concentration on 0.8% agarose mini-gel in 1xTBE buffer (0.09 M Tris-borate and 0.5 M EDTA) at 80 volts for 90 minutes with ethidium bromide staining. A total of 109 SSR adzuki bean primers were screened for good amplification and polymorphism[23,38]. PCR (polymerase chain reaction) reactions using established protocols[39,40] were carried out in a MJ Research PTC– 100™ Programmable Thermal Controller (MJ Research, INC, Waltham, MA, USA). The amplified PCR products were separated on 2.5 % agarose gel and stained with ethidium bromide. The generated molecular band data were used to determine the

[37] Roger SD & Bendich AJ (1988). Extraction of DNA from plant tissues, In Plant molecular Biology manual (eds) SB Gelvin, RA Schilperoot and DPS Verma (Dordrecht: Kluwer Academic Publishers) vol A6, pp 1-10.
[38] Li CD *et al* (2001). Determining genetic similarities and relatiomships among cowpea breeding lines and cultivars by microsatellite markers. Crop Sci. **41**: 189-197.
[39] Wang XW *et al* (2004). The development of SSR markers by a new method in plants and their application to gene flow studies in adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] *Theor Appl Genet* **109**: 352-360
[40] Somta P *et al* (2006). Development of an interspecific *Vigna* linkage map between *Vigna umbellata* (Thunb.) Ohwi and Ohashi and *V. nakashimae*) Ohwi) Ohwi and Ohashi and its use in analysis of bruchid resistance and comparative genomics. *Plant breeding* **125**: 77-84.

diversity parameters like number of alleles per locus, no of alleles per polymorphic locus, percentage of amplified primers, percentage of polymorphic markers (PPL), percentage of polymorphic alleles (PPA) and polymorphic information content of each primer[41].

Results

Agro-morphological characterisation and evaluation of ricebean accessions under core collection

Significant differences were observed between ricebean accessions across the sites for most agro-morphological traits including quantitative and qualitative traits (Tables 2.3.3 and 2.3.4). All the quantitative traits except days to maturity and seed per pod showed significant variation across the sites (p = >0.05; p = >0.01 and p = >0.001). These traits also showed a varied response to the growing environments, indicating a possible G x E interaction. For example, the days to flowering and 100 seeds weight varied significantly across study sites (Table 2.3.3). However, except for days to maturity and 100 seed weight there was no variation for most traits at Rampur (subtropical). Likewise at Kabre (high-hill), plants showed luxurious growth, and accessions significantly varied for height, leaf and seed traits and yield per plot. At Khumaltar (mid-hill), there was variation for most qualitative traits except for leaf measurement and seeds per pod (Table 2.3.3).

Table 2.3.3: Descriptive analysis and F-test of quantitative agro-morphological traits of core ricebean landraces across study sites , 2007 (number in parenthesis is the number of accessions examined)

Traits	**Diversity measures**	**NGLRP, Rampur (45)**	**HCRP, Kabre (46)**	**ABD, Khumaltar (45)**
Leaf length (cm)	Mean	10.0	10.4	8.4
	CV	0.07	0.22	0.20
	F-value	NS	***	NS
Leaf width (cm)	Mean	7.4	7.2	5.7
	CV	0.09	0.22	0.19
	F-value	NS	***	NS
Plant height (cm)	Mean	152.7	100.5	98.1
	CV	0.19	0.24	0.37
	F-value	NS	***	***
Days to flowering	Mean	116	94	89
	CV	0.04	0.07	0.10
	F-value	***	**	**
Days to maturity	Mean	150	NA	162
	CV	0.02		0.06
	F-value	NS		NS
Pod length (cm)	Mean	6.6	10.5	8.1
	CV	0.10	0.55	0.29
	F-value	NS	NS	**
Seeds per pod (No)	Mean	8	8	7
	CV	0.12	0.17	0.29
	F-value	NS	NS	NS
!00 seeds weight (g)	Mean	7.5	8.0	8.4
	CV	0.17	0.32	0.33
	F-value	**	**	*
Grain yield per plot (g)	Mean	503.5	220.2	332.4
	CV	0.64	1.03	0.64
	F-value	NS	*	**
Mean CV		**0.16**	**0.35**	**0.28**

NS = non significant; NA not available; * p = >0.05; **p = >0.01 and ***p = >0.001

The coefficient of variation ranged from 0.04-0.64% at Rampur; 0.07-1.03 at Kabre and 0.06-0.64 at Khumaltar (Table 2.3.3). Yield and yield components showed great variation between

[41] Weir BS (1996). Genetic data analysis II. Sunderland, MA, Sinauer

accessions, with the range of variation between the smallest to largest values moderate to high (data not shown). Table 2.3.4 shows the Shannon Weaver diversity indices for certain qualitative traits across sites. It ranged from 0.00 (pod shattering at Rampur) to 1.73 (seed colour at Kabre). Most traits were informative, with high diversity indices, and so useful to describe ricebean genotypes.

Table 2.3.4: Shannon Weaver diversity indices for qualitative agro-morphological traits. Core accessions, on-station, 2007 (number in parenthesis indicates the number of accessions examined)

Traits	**NGLRP, Rampur (45)**	**HCRP, Kabre (46)**	**ABD, Khumaltar (45)**
Hypocotyl colour	0.73	NA	072
Leaf shape	0.88	1.60	0.60
Leaf colour	0.89	1.03	1.00
Leaf pubescence	1.14	1.34	NA
Petiole colour	0.90	0.68	0.23
Growth habit	0.39	0.58	0.88
Growth pattern	0.10	0.00	0.28
Stem colour	1.11	1.18	0.40
Twining tendency	0.63	0.81	0.88
Raceme position	0.90	0.49	NA
Pod colour	1.33	NA	NA
Pod pubescence	0.62	0.00	NA
Pod suture colour	0.96	0.10	0.17
Pod shattering	0.00	NA	0.33
Seed colour	NA	1.73	1.11
Seed texture	NA	0.60	NA
Seed shape	NA	NA	0.86
Mean H'	**0.70**	**0.77**	**0.43**

NA = not recorded

Based on observed variations, NPGR 00008, NPGR 00015, NPGR 00076, NPGR 00194, NPGR 05364, NPGR 05420, NPGR 06756, LRGR 91, LRGR 99 and LRGR 111 performed best, and were selected for further evaluation for yield traits in mother trials in 2008. These accessions could be used in breeding programmes for landrace improvement. LRGR 91 and NPGR 00090 showed a susceptible reaction to rust at Kabre, but were high yielding. The accessions NPGR 05384 (Mugu), NPGR 05386 (Humla) NPGR 06591 (Mugu) and NPGR 07882 (Bajhang), the only determinate types, performed poorly in the field at all sites, and at Khumaltar became infected with diseases and insect pests at flower initiation and pod formation. Rust, cercospora leaf spot and web blight (*Rizoctonia* spp.) were the major diseases recorded and scored, based on damage at Khumaltar.

Agro-morphological characterisation and evaluation of non-core ricebean accessions
In this trial, 168 accessions (Annexe 3, Tables A3-1 – A3-4) were characterised and evaluated at three research stations, again under sole cropping. Tables 2.3.5 and 2.3.6 show the extent of variation. Coefficients of variation ranged from 0.02 (days to maturity) up to 1.76 (pod length), and averaged 0.17 at Rampur, 0.32 at Kabre, and 0.48 at Khumaltar (Table 2.3.5). A low CV was found for days to flowering and maturity across the study sites.

Accessions exhibited a wide range of variation for most traits, with an average diversity index of 0.89 (Kabre), 0.92 (Khumaltar) and 0.93 (Rampur) (Table 2.3.6). The least diversity was for growth pattern (0.24, Kabre) and highest (1.71) for seed colour (Khumaltar).

NPGR 00005, NPGR 00184, NPGR 00191, NPGR 05422, NPGR 05424 (Kabre, Table A3-3); NPGR 05367, NPGR 05381, NPGR 05383, NPGR 05425, NPGR 08381 (Rampur, Table A3-2) and NPGR 00189, NPGR 05372, NPGR 05374, NPGR 05410, LRGR 87, LRGR 113, LRGR 123, LRGR 133, LRGR 139, LRGR 143, LRGR 153, (Khumaltar, Table A3-4) were selected for further evaluation in an observation nursery in 2008, and could be used in breeding programme.

Table 2.3.5: Descriptive analysis of quantitative agro-morphological traits in the non-core ricebean landraces across study sites, 2007 (number in parenthesis indicates the number of accessions examined)

Traits	**Diversity measures**	**NGLRP, Rampur (43)**	**HCRP, Kabre (47)**	**ABD, Khumaltar (168)**
Leaf length (cm)	Mean	10.1	12.1	8.2
	CV	0.12	1.05	0.24
Leaf width (cm)	Mean	7.5	7.4	5.7
	CV	0.13	0.15	0.24
Plant height (cm)	Mean	151.1	100.2	85.2
	CV	0.27	0.25	0.37
Days to flowering	Mean	117	94	84
	CV	0.04	0.09	0.22
Days to maturity	Mean	150	143	159
	CV	0.02	0.06	0.11
Pod length (cm)	Mean	6.6	9.4	17.6
	CV	0.12	0.13	1.72
Seeds per pod (No)	Mean	8	8	7
	CV	0.20	0.23	0.23
!00 seeds weight (g)	Mean	7.3	8.2	7.9
	CV	0.14	0.31	0.38
Grain yield per plot (g)	Mean	694.1	303.2	367.5
	CV	0.62	0.65	0.84
Mean CV		**0.17**	**0.32**	**0.48**

In this study, growth habit, duration of flowering, seed colour and size were important traits that showed high phenotypic diversity. The findings indicate a great scope for improving ricebean for desired traits through breeding, but further evaluation and selection of key genotypes is still required

Table 2.3.6: Shannon weaver diversity indices of quantitative agro-morphological traits in the non-core ricebean landraces across study sites, 2007 (number in parenthesis indicates the number of accessions examined)

Traits	**Rampur (43)**	**Kabre (47)**	**Khumaltar (168)**
Hypocotyl colour	0.57	NA	0.63
Leaf shape	0.69	1.15	1.33
Leaf colour	1.19	0.65	NA
Leaf pubescence	1.05	0.89	NA
Petiole colour	1.17	1.09	NA
Growth habit	0.54	0.39	1.02
Growth pattern	NA	0.24	0.88
Stem colour	1.21	1.11	0.54
Twining tendency	0.80	1.05	1.03
Raceme position	0.97	0.38	NA
Pod pubescence	0.68	NA	NA
Pod suture colour	1.32	NA	0.06
Pod shattering	NA	NA	0.40
Seed colour	NA	2.11	1.71
Seed texture	NA	0.76	NA
Seed shape	NA	NA	1.42
Mean	**0.93**	**0.89**	**0.92**

Identification of polymorphic loci

Parameters for genetic variation observed for 27 ricebean accessions consisting of different seed morphotypes and collections from different agro-ecosystems in Nepal are summarized in Tables 2.3.7 and 2.3.8. Of the 85 bands amplified by 49 primers in ricebean (excluding adzuki bean), 63 (74 %) were polymorphic with an average of 2.1 polymorphic bands per primer. This is close to the average value of 2.3 polymorphic bands per primer computed for all accessions, including the adzuki bean check (Table 2.3.7). Similarly, the average number of amplified bands per primer was 1.7 for ricebean, and 1.9 for all accessions including adzuki bean. The percentage of polymorphism as PIC ranged from 7 % (CEDG018) to a maximum of 67 % (CEDG073), with an average of 24 %. Likewise the Shannon Weaver diversity index ranged from 1.28 (CEDG073) down to 0.17 (CEDG007, CEDG021 and CEDG141), with an average of 0.41 for the ricebean accessions (Table 2.3.8)

Table 2.3.7: Summary of SSR Diversity values calculated for ricebean accessions and all samples

Diversity parameters	**Ricebean**	**All accessions including adzuki bean**
Number of accessions consisted	27	29
Number of SSR primers screened	109	109
Number of primers amplified	49	49
Number of monomorphic primers	19	14
Number of polymorphic primers	30	35
Percent of polymorphic primers	61	71
Total number of alleles (bands) observed	85	93
Number of alleles /primer	1.7	1.9
Total number of polymorphic alleles (bands)	63	79
Number of alleles/polymorphic primer	2.1	2.3
Percent of polymorphic alleles (bands)	74	85
Polymorphic information content (PIC)	0.26	0.24
Shannon Weaver index (SW)	0.45	0.41

All the polymorphic primers from Table 2.3.2 contained AG repeats, except for CEDAAG002, which had AAG repeats, and CEDG044 which had GT(AT)AG repeats. These primers belong to linkage groups 1, 2, 3, 6, 8, 10 and 11 of the ricebean genome. These primers explained the variation at DNA level among ricebean accessions, and also between adzuki bean and the ricebean accessions.

Table 2.3.8: PIC and Shannon indices of 13 polymorphic adzuki bean SSR primers with higher PIC than the average value 0.24 of ricebean accessions

Primers	**Linkage**	**Alleles (bands)**	**PIC values**		**Shannon index**	
			All accessions	Ricebean	All accessions	Ricebean
CEDAAG002	2	3	0.66	0.65	1.30	1.21
CEDG015	1	3	0.48	0.43	0.79	0.62
CEDG073	8	4	0.68	0.67	1.45	1.28
CEDG286	2	2	0.36	0.28	0.54	0.45
CEDG294	3	3	0.35	0.30	0.71	0.63
CEDG232	9	2	0.30	0.24	0.48	0.41
CEDG253	8	2	0.28	0.24	0.45	0.40
CEDG044	11	3	0.42	0.30	0.83	0.63
CEDG178	1	2	0.64	0.65	1.06	1.07
CEDG037	6	3	0.59	0.58	0.52	0.45
CEDG195	6	2	0.36	0.32	0.38	0.26
CEDG134	10	2	0.36	0.40	0.54	0.59
CEDG050	2	3	0.35	0.30	0.63	0.21

A set of 35 SSR markers gave good amplification of DNA in ricebean. Among the primers evaluated, only 13 were found to be polymorphic, with an average PIC of 0.24, and these could be used to explain diversity in a ricebean germplasm collection. These primers were distributed across the genome, and constituted di-nucleotides of AG. They will be used in subsequent assessment of molecular marker diversity in the ricebean landraces evaluated and selected in 2007.

2.4 Workpackage 4: Germplasm characterization and adaptation

2.4.1 Project objectives and major achievements during the reporting period

Gramin Vikas Trust (GVT, Partner 4) leads WP4: **To characterise the germplasm for phenological traits and suitability for a range of diverse environments and cropping systems**. Assam Agricultural University (AAU, Partner 6) and Chaudhary Sarwan Kumar Krishi Vishwavidyalaya**,** Palampur, Himachal Pradesh (CSKHPAU, Partner 5) are the other two participants in WP 4 in India, and Local Initiatives fro Biodiversity, Research and Development (LI-BIRD, Partner 8) in Nepal. The work package has the following objectives:

- To characterise ricebean germplasm using participatory approaches and identify that best meet farmers' needs in terms of morphology, flowering time and grain quality traits in Nepal and India.
- To analyse and understand farmers preferred traits for ricebean in India and Nepal
- To test selected accessions in Mother and Baby trials.

Various activities conducted in this work package have been summarized in Table 1.

Table 2.4.1. Summary of objectives and work performed during reporting period

Objectives	Worked performed	Deliverables
4.1 Identify a set of accessions that best meet farmers' needs in terms of morphology, flowering time and grain quality traits.	Accessions from different districts of India by respective partners have been collected and evaluated during *kharif* 2007 (rainy season).	Sufficient seed samples are available for further germplasm evaluation.
4.2 Test selected accessions in Mother and Baby trials.	Promising landraces have been identified and evaluated in *Kharif* 2007 at farmer's field through mother and baby trials and phonology, yield and yield components related data was collected.	Mother and baby trials have been conducted as per protocol developed for mother and baby trials. Seeds of all the promising entries are available for mother and baby trials for the next year.
4.4 Analyze data across locations and years.	Data of mother and baby trials have been recorded location wise and analyzed as per statistical methodology applicable.	Quantitative and qualitative data of mother and baby trials has been analysed.

2.4.2. Summary of work in year 1

In the first year, farmer-preferred trait analysis was carried out in both India and Nepal, in the same districts as the germplasm collection in WP2 and using focus-group discussions with mixed groups of between 10 and 20 male and female farmers. Standard protocols developed by the project partners were used to collect the data, and included the assessment of organoleptic traits such as taste and cooking quality. Attempts have been made to exchange germplasm between the two countries, but this has so far not proved possible. Of the collected and evaluated germplasm in WP2, the most promising accessions that meet farmers' requirements have been identified for sowing in mother and baby trials in 2007.

Ricebean did not compare well with other locally-grown legumes: out of eight crops, farmers in both Nepal and India preferred black gram (*Vigna mungo*) and kidney bean (*Phaseolus vulgaris*), with ricebean placed seventh, ahead of only lentil (*Lens culinaris*).

All parts of the crop were used, for a range of purposes including *dal* (soup), as a fresh vegetable and snack, as a livestock forage and as a green manure. Food was the most important use, followed by fodder production and green manuring. Positive and negative traits were identified for the various uses in both countries. Large-seeded determinate and synchronous varieties were preferred for food production, and late-maturing indeterminate types with soft palatable herbage for fodder and green manure. Early maturing varieties were not favoured as their maturity coincided with the peak rainy season, and they also had low yields and small grains. Pest and disease resistance, and suitability for intercropping or mixed cropping were regarded as less important, as was tolerance to high rainfall during flowering.

Overall, in Nepal preference was given to the landrace *Seto Thulo* (white bold [large]) while in India the three preferred types were all bold-seeded. It was felt important for any breeding work to include organoleptic traits.

2.4.3 Workpackage progress of the period

In Nepal, mother and baby trials were conducted at the same two sites in Gulmi as for the germplasm evaluation. The mother trials grew eight accessions each. There has not been enough seed for a full trial set up – much of the problem of seed availability had been due to heavy rain at crucial times in the growing season.

In India, GVT conducted 27 mother and forty baby trials at four locations in MP. In Himachal Pradesh, we conducted sixteen mother trails, with six entries from HP, Assam, and MP. In addition, ten baby trials are under way to evaluate three preferred genotypes (one each from Assam, HP and MP). These are at two locations, with five farmers at each site. In Assam, Mother and baby trials were carried out in two villages. The mother trials had six entries, with three trials per village, and the baby trials three entries with fifteen trials per village. Villages were selected for contrasting socio-economic conditions, and past experience of the crop. The AAU varieties are clearly late flowering (generally towards the end of September).

Seed multiplication has been carried out to ensure sufficient seed is available for the next season.

A trial in HP assessed the effect of priming seed with a phosphate solution, to overcome problems of low P availability in the crop. This showed a clear advantage in terms of crop emergence for primed v non-primed seed, and further data will be provided at the end of the season.

Mother trials intercropped with maize

Eight accessions identified in the on-farm evaluation in 2006 as having good potential were selected for mother trials (Table 2.2.3). The mother trials were conducted in an intercropping system with maize (Annexe 2, Fig 1).

Six mother trials, three at Darbar Devisthan and three at Simichaur VDC, both in Gulmi, Nepal were conducted on farmers' fields using the farmers' usual inputs and agronomic practices, with each farmer having one trial. The farmer-perceived soil fertility status of the trial sites, and the nutrient status found from laboratory analysis, are presented in Table 2.2.4 (data for two of the trials are not recorded).

Table 2.2.3. Accessions tested in mother trials on-farm in Gulmi (All collected by LI-BIRD)

Accession	Districts collected	Local name
LRGR91		
LRGR117	Gulmi	Jhilinge
LRGR111	Gulmi	Jhilinge
LRGR42	Surkhet	Siltung
LRGR103	Palpa	Jhilinge
LRGR75	Pyuthan	Raiyans
LRGR44	Surkhet	Siltung
LRGR152	Kavre	Masyang

Table 2.2.4. List of cooperating farmers and soil fertility status of trial site

Farmer's name	Location	Farmer perceived soil fertility	Soil fertility status (Lab analysis)								
			pH	% OM	% N	Avail. P ppm	Avail. K ppm	% sand	% silt	% clay	Soil type
Jit Bahadur Thapa	Darbar Devisthan	Poor	6.10	2.87	0.15	5.55	138	67.5	30	2.5	SL
Top Bahadur Thapa	Darbar Devisthan	Poor	6.10	2.87	0.15	5.55	138	67.5	30	2.5	SL
Dornacharya Panthi	Darbar Devisthan	Medium									
Prem Raj Marasini	Simichaur	Medium	6.87	3.65	0.19	4.55	416	57.5	30	12.5	SL
Jaya Prasad Aryal	Simichaur	Poor	6.20	3.56	0.20	59.0	94.9	72.5	25	2.5	SL
Risiram Aryal	Simichaur	Medium									

SL= sandy loam

Farmers' criteria to classify soil (high, medium or low fertility) are mainly based on colour, while they rate soil proprieties based on their working knowledge of soil, and the production of the crop they get from that soil. Black soil is thought to be the most fertile, red medium, and brown or grey soils are thought low in fertility. Poor soils were low in organic matter, phosphorus and potassium in the laboratory analysis (Table 2.2.4), and in general the results from the laboratory agreed with the farmers' classification, indicating that farmers' criteria to describe soil fertility in Nepal are quite sensible.

In India, mother trials were conducted at large scale by all the partners in their respective project areas. Each trial contained six entries, three from Assam, two from Himachal Pradesh (HP) and one from GVT (Table 2.4.3).

Table 2.4.3: Experimental material used in the mother trials

Genotype	Source of seed
JR-1	GVT
RBD-1	Himachal Pradesh
RBC-2	Himachal Pradesh
JCR-07- 07	Assam
JCR-07-15	Assam
JCR-07-16	Assam

In western Madhya Pradesh (MP), 27 mother trials were conducted during *rabi* (rainy season) by GVT in six villages in Meghnagar, Alirazpur, Katthiwara and Barwani, of which 15 were successfully harvested: the others were badly damaged by heavy rain. In HP, of 17 mother trials sown, 16 were successfully conducted (two in villages Trilokpur and Khundian and one at the university farm) and one was lost due to grazing by stray animals. In Assam, seven mother trials were conducted, at the AAU main centre, and at Danisapori and Rongkhangthir in Karbi Anglong District. The trials were sown in plots of 2.7 x 4 m, with 4 meter row length and row spacing of 45 cm in randomized block design. Each farmer was treated as one replication.

Results

3.3.2 Mother trials in Nepal:

In 2007, eight selected genotypes of ricebean were evaluated in mother trials in Gulmi for agro-morphological and agronomic traits and for farmers' ranking. The accessions varied significantly for four traits, flowering and maturity periods, plant height and pod length (Table 2.2.7). The difference between accessions for days to flowering was less than for days to maturity (103 – 108 days, v 156 – 183 days).

Table 2.2.7. Mean performance of different agro-morphological and agronomic traits of rice bean in mother trials conducted in Gulmi, Nepal in intercropping system, 2007

Entry no	Days to emergence	Days to 50% flowering	Plant height (cm)	Pod length (cm)	Days to maturity	No of fertile pods/plant	No of seeds/pod	Grain yield (t /ha	Rank
LRGR91	7	104	160	8.2	160	374.1	6.5	0.42	1
LRGR117	8	103	188	8.7	161	520.2	6.9	1.00	5
LRGR111	8	106	208	8.5	158	488.2	7.0	0.86	7
LRGR42	7	106	219	10.9	169	349.8	6.5	0.51	1.7
LRGR103	7	107	240	8.7	161	512.3	6.8	1.39	8
LRGR75	8	107	179	9.1	156	497.5	8.1	0.84	4
LRGR44	7	107	162	9.9	184	180.5	6.3	0.74	6
LRGR152	7	108	195	9.2	178	541.9	7.4	0.91	3
Mean	8	106	193	9.1	165	443.7	6.9	0.82	4.3
F test	ns	**	*	*	*	ns	ns	ns	*
CV %	27	4.70	13.9	7.7	2.6	59.1	18.3	52.60	2.7
l.s.d. (0.05)	1.45	1.90	48.97	1.38	14.2		1.2	0.58	0.37

** Highly significant at p<0.01, * significant at p<0.05, ns= non significant

Plant height also varied greatly, from 160 – 240 cm, while variation for pod length was moderate (8 – 11 cm). Differences for most yield components, and for yield, were statistically non-significant; although entries LRGR103 and LRGR117 showed a slight yield increase. LRGR 103 and LRGR 111 were the most preferred genotypes in farmers' ranking because of a good combination of medium maturity, number of pods per plant and good yield.

The mother trials were designed as a dispersed RCBD with farmers as replicates, and were conducted in two locations with different fertility status. Environmental factors such as the fertility status of the trial sites, and effects of their location, are likely reasons for the high coefficient of variation for numbers of fertile pods per plant (59.1%) grain yield (52.6%) and numbers of seeds per pod (18.3 %) (Table 2.2.7).

Table 2.4.4. Mean data of mother trials conducted by GVT during *Kharif* 2007

Observation/Variety	JR-1	RBD-1	RBC-2	JCR-07-07	JCR-07-15	JCR-07-16
Seedling vigour[1]	F	F	F	M	M	M
Days to 50% flowering	86.72	55.02	58.32	94.95	97.35	97.38
Plant growth habit[2]	T	T	T	SE	SE	SE
Number of pods per plant	37.10	38.40	37.85	26.95	30.38	29.76
Days to maturity	145.22	116.36	120.19	154.72	157.56	156.50
Pod length (cm)	4.74	4.48	4.38	4.16	4.31	4.30
Number of seeds per pod	4.82	4.98	4.79	3.65	3.95	4.21
100 seed weight (g)	5.84	5.90	5.81	17.86	6.32	6.13
Plant height (cm)	110.79	98.05	96.03	69.88	72.39	69.43
Grain Yield (kg/ha)	216	239	213	163	149	141

CV % 18.68

$LSD_{0.05}$ 54.26 kg/ha

[1]M=Medium, S=Slow, F=Fast
[2]E=Erect, SE=semi erect, T= trailing

In the trials conducted by GVT, RBD-1 had the highest yield (239 kg ha^{-1}) followed by JR-1 (216 kg/ha), RBC-2 (213 kg/ha) and JCR-07-07 (163 kg/ha) (Table 2.4.4). RBD-1 and JR-1 were significantly better than the other lines except RBC-2. RBD-1 wad also earliest to flower (55.0 days) and to mature (116.4 days), followed by RBC-2 (58.3 and 120.2 days) and JR-1 (86.7 and 145.2). These three entries also had the greatest number of pods per plant and were tallest. JCR-07-07 had the highest 100-seed weight (17.9 g). Three lines (JR-1, RBD-1 and

RBC-2) were of a trailing type, and three (JCR-07-07, JCR-07-15 and JCR-07-16) were semi-erect. All entries in all trials showed indeterminate maturity.

Table 2.4.5 shows matrix rankings from farmer evaluations of the trials. Overall, JR-1, RBD-1 and RBC-2 were preferred by farmers over the other genotypes.

Table 2.4.5: Mean data of matrix ranking of mother trials.(GVT)

Characters	JR-1	RBD-1	RBC-2	JCR-07-07	JCR-07-15	JCR-07-16
Seedling vigour	5	5	5	3	3	3
Days to 50% flowering	3	4	4	2	2	2
Plant growth habit	3	3	3	4	4	3
Number of pods per plant	4	4	3	2	2	2
Days to maturity	3	4	4	2	2	2
Type of maturity	3	3	3	3	3	3
Pod length	4	4	4	3	3	3
Number of seeds per pod	4	4	4	2	2	2
Seed size	3	3	3	5	3	3
Grain yield	4	4	3	3	3	3
Dry straw yield	-	-	-	-	-	-
Total	**36**	**37**	**36**	**29**	**27**	**26**

In Assam, the highest yield was from JCR-07-7 followed by JCR-07-15 and JCR-07-16. Yield attributing characters were also higher in these entries. JR-1 was the latest to mature, and JCR-07-, JCR-07-15 and JCR-07-16 were medium maturity, while RBD-1 and RBC-2 matured early. Highest 1000 seed weight was also found in JCR-07-7.

Table 2.4.6 Mean data of matrix ranking of mother trials in Assam

Characters	Name of Entry					
	RBC-2	**JCR-07-16**	**JR-1**	**JCR-07-15**	**RBD-1**	**JCR-07-7**
Seedling vigour	4	5	3	5	4	5
Days to 50% flowering	4	3	2	3	4	3
Plant growth habit	3	4	3	4	3	4
Number of pods per plant	2	3	4	3	2	3
Days to maturity	4	3	2	3	4	3
Type of maturity	4	3	2	3	4	3
Pod length	3	4	3	4	2	4
Number of seeds per pod	4	3	4	3	3	3
Seed size	3	4	3	4	3	5
Grain yield	2	4	3	4	2	5
Dry straw yield	-	-	-	-	-	-
Total	**33**	**46**	**29**	**36**	**31**	**38**

In HP, the entries from Assam and GVT failed to produce any seed because they were very late in flowering. After October the temperature in the area fell sharply, and none of this material bore any pods – JR-1 from GVT even failed to flower. The mean performance of the six genotypes in Trilokpur and Khundian, and also combined over locations, is shown in Table 2.4.7.

Table 2.4.7: Mean performance of mother trials at Trilokpur, Khundian, and over both locations

Entries	Plant height (cm)			Dry biomass/ plant (g)			Days to 50% flowering		
	Trilokpur	Khundian	Mean	Trilokpur	Khundian	Mean	Trilokpur	Khundian	Mean
RBD-1	155.4	132.2	143.8	4.72 (4.37)	4.24 (3.93)	4.48 (4.15)	65.9	67.8	66.9
RBC-2	159.3	131.4	145.4	4.75 (4.40)	4.73 (4.38)	4.74 (4.39)	69.3	69.8	69.6
JR-1	140.0	120.7	130.3	4.79 (4.44)	4.97 (4.60)	4.88 (4.52)	-	-	-
JCR07-7	136.9	129.3	133.1	6.33 (5.86)	5.19 (4.81)	5.76 (5.33)	115.6	116.0	115.8
JCR07-15	166.2	141.9	154.1	4.54 (4.21)	4.74 (4.38)	4.64 (4.30)	115.1	116.2	115.7
JCR07-16	168.7	140.3	154.5	5.46 (5.05)	5.33 (4.93)	5.39 (4.99)	115.0	115.8	115.4
Grand Mean	154.4	132.6	143.5	4.72	4.51	4.61	96.2	97.1	96.7
CD (5%)	8.50	10.89	6.77	0.80	5.80	2.89	1.70	1.43	1.08

Figures in parenthesis are the values in t ha^{-1}

The range of plant height varied from 136.9 cm (JCR-07-7) to 168.7 cm (JCR-07-16) in Trilokpur, and from 120.7 cm (JR-1) to 141.9 cm (JCR-07-15) in Khundian. The range of dry biomass per hectare were 4.21 t ha^{-1} (JCR-07-15) to 5.86 t ha^{-1} (JCR-07-7) in Trilokpur, and 39.3 (RBD-1) to 4.98 t ha^{-1} (JCR-07-15) in Khundian. On average, RBD-1 had the lowest production (4.15 t ha^{-1}) and JCR-07-7 the highest (5.33 t ha^{-1}). The days to 50% flowering ranged from 65.9 (RBD-1) to 115.6 day (JCR-07-7) in Trilokpur, and from 67.8 (RBD-1) to 116.2 days (JCR-07-15) in Khundian, with the fasted overall being RBD-1 (66.9 days) and the slowest to flower JCR-07-7 (115.8 days). Genotypes JCR-07-7, JCR-07-15 and JCR-07-16 were particularly slow to flower, while JR-1 did not flower at all. Of the lines that produced seed, RBD-1 gave a higher yield than RBC-2 in both locations.

Matrix ranking

JR-1, JCR-07-07, JCR-07-15 and JCR-07-16 failed to produce seed under conditions in HP as they were late in maturity and did not flower until after the completion of the crop season. In consequence, they were not liked by farmers. Of the local genotypes, RBD-1 was preferred over RBC-2, particularly for its better yield (Table 2.4.8).

Table 2.4.8: Mean data of matrix ranking of mother trials (Palampur)

Characters	**JR-1**	**RBD-1**	**RBC-2**	**JCR-07-07**	**JCR-07-15**	**JCR-07-16**
Seedling vigour	5	5	5	3	3	3
Days to 50% flowering	1	6	6	-	-	-
Plant growth habit	5	4	4	4	4	3
Number of pods per plant	-	6	5	-	-	-
Days to maturity	-	5	5	-	-	-
Type of maturity	-	3	3	-	-	-
Pod length	-	5	4	-	-	-
Number of seeds per pod	-	5	4	-	-	-
Seed size	-	4	4	-	-	-
Grain yield	-	6	4	-	-	-
Dry straw yield	-	3	3	-	-	-
Total	**11**	**52**	**47**	**7**	**7**	**6**

Baby trials

A large number of baby trials were conducted with and managed by farmers in many different locations These were designed to test all combinations of three genotypes, JR-1 from GVT, RBD-1 from HP, and JCR-07-7 from Assam. Farmers were given assistance with plot layout and sowing but managed the plots using their own methods. The trials were sown at a row spacing of 45 cm, and 250 g seed of each line was provided to the farmers. GVT carried out 36 trials in 13 villages in five districts, sown in late June 2007. In Assam, AAU conducted thirty trials in two villages (Danichapon and Rongkangthir) in two districts (Dergaon and Diphu respectively), sown in mid-July, while in HP twelve villages were used in two districts. From sowing to maturity, project staff visited each farmer's field and conducted a preference analysis in which farmers were asked to rank each variety using plant type, maturity, grain size, grain yield, grain colour, taste, fodder palatability, cooking quality. They were then asked to indicate positive and negative characteristics of varieties, and which varieties they would continue to grow next year. Yield data were collected after harvest. Table 2.4.11 shows the cultural techniques and farmer preference data from these trials.

The trials in GVT were initially analysed using analysis of variance as a randomised block, and this showed significant difference between varieties for grain yield. Entries were also subjected to paired *t*-tests, and this failed to show significant differences between genotypes for grain yield. In Assam, the highest yields and 100-seed weights were from JCR-07-7. In HP, the trials were conducted successfully in 5 villages. Analysis of variance concluded that there was significant difference between varieties and villages for grain yield. At both sites in HP, farmers preferred short duration dwarf varieties that allowed the timely sowing of a following crop of vegetables, wheat, or pulses. On the basis of one year of testing at Palampur, RBD-1 was preferred for its plant type, early to medium maturity, grain yield, grain colour, taste, fodder palatability etc. Farmers rejected JR-1 & JCR-07-16 because of their late maturity, although they liked them for their fodder palatability.

Table 2.4.9: Mean data from baby trials conducted during *kharif* 2007 by GVT

	Location								
	GVT			Assam			HP		
Particulars	JR-1	RBD-1	JCR-07-7	JR-1	RBD-1	JCR-07-7	JR-1	RBD-1	JCR-07-7
Soil type	Black / stony / red	Black / stony / red	Black / stony / red	Sandy loam, sandy clay	Sandy loam, sandy clay	Sandy loam, sandy clay	Clay loam	Clay loam	Clay loam
Organic manure*	Yes/no	Yes/no	Yes/no	Yes	Yes	Yes	Yes/no	Yes/no	Yes/no
Ploughing	2-3	2-3	2-3	2/3	2/3	2/3	2-3	2-3	2-3
Green manuring	No	No	No	No	No	No	No	No	No
Chemical fertilizers	No	No	No	No	No	No	No	No	No
Insect attack*	Yes / no	Yes / no	Yes / no	No	No	No	Yes	Yes	-
Disease appearance	No	No	No	No	No	No	No	No	No
Insecticide used*	No/yes	No/yes	No	No	No	No	Yes	Yes	-
Herbicide used	No	No	No	No	No	No	No	No	No
Flowering	Medium	Early	Late	Late	Early	Medium	Early	Late	V late
Pods	Same	Same	Less	More	Same	Same	More	None	None
Pod size (small, medium, large)	S	S	L	S	S	L	M	-	-
No. of seed/pod	More	More	Less	More	More	Less	More	-	-
Shattering	Yes	Yes	Yes	No	No	No	Yes	-	-
Growth habit	I	I	I	I	I	I	I	I	I
Grain type (bold, medium	M	M	B	M	M	B	M	-	-
Maturity (early, medium, late)	M	E	L	L	E	M	M		
Fodder quality (good, excellent)	G	G	G	-	-	-	G	E	E
Average yield (g per 250 seed)	1114.4	1074.8	607.5	716.1	670.6	1058.6	1035.6	-	-
Overall liking	Good	Good	Good	Good	Good	Good	Good	Good†	Good†

*In some trials there was an attack of insect pests, use of insecticide and use of organic manure (FYM).
†As fodder

Winter (*rabi*) ricebean

Following discussion in the second annual meeting, ricebean was planted in the *rabi* season (winter) at Kattiwara, Jhabua (MP) and Dahod (Gujarat). Two varieties, BRS-2 and JR-1 were tested in farmers' fields, but no seed set was observed.

Effect of seed priming of ricebean in maize + ricebean intercropping system

An additional experiment was carried out to investigate the effect of seed priming (soaking seed overnight before drying and sowing the next day). Ten treatment combinations which included soaking in water, and soaking in a solution containing phosphate, were tested on the experimental farm of CSKHPKV in a randomized block design with three replicates during *kharif* 2007. Each treatment comprised 6 rows of 4 m length with row to row spacing of maize 60 cm. The plant to plant spacing of ricebean was 15 cm, and of maize 20 cm.

Table 2.4.10: Effect of seed priming treatments on yield of maize and ricebean

Treatments	Maize yield (t ha^{-1})	Ricebean yield (q/ha)
No P - Sole maize	1.23	-
No P + maize + ricebean (seed not primed)	1.35	0.42
No P + maize + ricebean (primed with water)	1.40	0.39
No P + maize + ricebean (primed with 0.1 % P)	1.35	0.47
No P + maize + ricebean (primed with 1 % P)	1.29	0.53
Recommended P + sole maize	1.83	-
Recommended P + maize + ricebean (seed not primed	1.96	0.52
Recommended P + maize + ricebean (primed with water)	2.06	0.62
Recommended P + maize + ricebean (primed with 0.1 % P)	1.99	0.72
Recommended P + maize + ricebean (primed with 1 % P)	2.12	0.69

The priming of ricebean seed with different treatment did not show any significant effect on emergence. However, P application had significant effect on yield of both the crop components (Table 2.4.10). Seed priming of ricebean with P also shows promising results in relation to better seed yield of this crop.

2.5 Workpackage 5: Nutrition and health

2.5.1 Objective

The objective of WP 5 is *"to assess the potential impact of enhanced pulse availability on local human nutrition"*. The WP is led by Bergen, with field work in Asia carried out by GVT, CSKHPKV AAU, NARC and LI-BIRD. The rationale has been to study the relative role of ricebean, actual and potential, in areas with different staple crops and with and without ricebean present in their dietary system. In order to make the analysis manageable, the study is focused on women of reproductive age, a group which hypothetically could be more at risk of inadequate nutrition than males and other age groups.

2.5.2. Summary of work in year 1

A comprehensive literature survey on the nutritional qualities of ricebean, the state of the art of nutritional survey design, and the extent of malnutrition in the region was undertaken by the University of Bergen (UB). The survey has to some extent reduced the need for chemical analyses, although some additional analyses will have to be undertaken. It also showed that the suggested strategy of WP5 was justified, as no comprehensive studies in the region have been published on diet composition and adequacy, and the relative contribution of pulses in the diets is largely unknown. The dietary survey will provide new scientific knowledge essential to FOSRIN but also generally publishable.

In extension of the kick-off meeting, a preliminary field appraisal was carried out in Ilam district, Eastern Nepal. This provided very valuable information on different types of cultivation as well as the integrated role of ricebean in the farming system. Knowledge on preferential traits was collected as part of WP4, providing a good basis for the design of more extensive field efforts in mid-2007.

An intensive training session was held in Palampur and attended by the Asian partners. The purpose was to train project staff in the various techniques that would be needed to carry out the dietary surveys, and to standardise the methodologies. The training workshop was followed by a practical session in the field.

2.5.3 Workpackage progress of the period

A questionnaire and standard food models were developed, and the survey carried out on three aspects (baseline diets; 24 hours recall and food frequency) at three different periods over six months in 2007. The selection of sites was made with the consensus of the project team and local informants such as VDCs in Nepal, using criteria such as area under ricebean cultivation, agro-ecological and cropping diversity, ethnic diversity and the accessibility. In Nepal, surveys were carried out in cooperation with NARC in Dolakha (high-hill, central Nepal) and Dhankuta (mid-hill, Eastern Nepal), and with LI-BIRD in four areas of Gulmi District. In India, surveys were done at locations in Himachal Pradesh (with CSKHPKV), and in Assam (with AAU). This gives a good variety of staples (rice, wheat, maize, millet) and a rich data set for comparative analysis. The time space over which the data were collected was planned to include pre-monsoon or early monsoon as well as post-harvest situation, aimed at study periods of presumed low as well as better food supply.

The dietary recalls were done by four teams, collecting data from each 200 women. Locally selected women enumerators and project staff of the site were used. The last data have been collected in the first months of 2008. The survey sheets have been assessed and 'cleaned', and the present situation is that all survey sheets have been punched, using the WorldFood2 standard programme. The analysis is expected to be carried out over the following months, a slight delay compared to the original plan, but not putting the deliverables from WP5 at any risk.

Table 2.5.1: Example of nutrition data entered in WorldFood2 format

Country		
grp1		
grp2		
grp3		
tea		
warning	1	
fcode		
name_engli	ricebean	
enerc_kcal	327	Duke 1981
a_kcal	0	
procnt	18	Kaur & Kapoor 1992
a_protein	0.0	
mfp_protein	0.0	
fat	0.49	Kaur & Kapoor 1992
chocdf	59.0	Kaur & Kapoor 1992
fasat	0.3	Kaur & Kapoor 1992
fams	0.0	Kaur & Kapoor 1992
fapu	0.2	Kaur & Kapoor 1992
chole	0	Eurofins 2007
fib	7.0	Kaur & Kapoor 1992
sucs	2.7	Revilleza et al. 1990
phytac	2033	Kaur & Kapoor 1992
vita	0	Eurofins 2007
a_vita	0	
vitd	0	Eurofins 2007
vite	0	Eurofins 2007
vitc	1	Kaur & Kapoor 1992
thia	0.49	Duke 1981
ribf	0.31	Kaur & Kapoor 1992
nia	2.4	Duke 1981
vitb6	0.04	Eurofins 2007
fol	14	Eurofins 2007
vitb12	0.05	Eurofins 2007
pantac	0.12	Eurofins 2007
ca	264	Mohan & Janardhanan 1994
p	124	Mohan & Janardhanan 1994
mg	73	Mohan & Janardhanan 1994
k	2875	Mohan & Janardhanan 1994
na	6	Mohan & Janardhanan 1994
fe	6.7	Kaur & Kapoor 1992
mfp_fe	0.0	
zn	3.1	Kaur & Kapoor 1992
cu	1.46	Kaur & Kapoor 1992
mn	2.70	Kaur & Kapoor 1992
trp	0	n.d. - Mohan & Janardhanan 1994
thr	571	Mohan & Janardhanan 1994
ile	1002	Mohan & Janardhanan 1994
leu	1387	Mohan & Janardhanan 1994
lys	1142	Mohan & Janardhanan 1994
met	448	Mohan & Janardhanan 1994
cys	0	Trace, Mohan & Janardhanan 1994
phe	894	Mohan & Janardhanan 1994
tyr	541	Mohan & Janardhanan 1994
arg	1132	Mohan & Janardhanan 1994
his	689	Mohan & Janardhanan 1994

With the aim of analysis of the specific nutritional value of ricebean, a full range of nutrient figures has been compiled in WorldFood2 format (Table 2.5.1)). The majority the values have been compiled from existing literature. It can be expected that different varieties of ricebean grown under various conditions will exhibit considerable variation. Therefore, the sources have been selected according to what appears to be consensus in the literature, and from what can be assumed as the best, peer reviewed literature. With respect to some nutrients, notably vitamins, laboratory analysis was carried out in Norway. A very brief summary of the knowledge on nutritional aspects is that ricebean will be as follows:

- Moderate raw protein content but with high digestibility
- Very beneficial amino acid composition for human consumption
- Content of vitamins and minerals comparable to other pulses
- Low fat content dominated by unsaturated lipids
- No specific problems concerning toxic or allergenic substances
- No unusual anti-nutrients

Initial results suggest that ricebean has a reasonable iron and zinc content, but the low levels of the crop in diets mean there was no significant benefit in terms of these nutrients at present.

Dissemination activities

In HP, feedback from the dietary survey feedback showed that ricebean was mainly consumed as *Dhal*, and was not greatly relished because of its bland taste. However, when preparation are made by adding some other ingredients like mango powder its popularity increases. Villagers in Trilokpur and Khundian (Kangra district) were asked to suggest methods for using ricebean in the preparation of value added products.

Table 2.5.2: Products prepared and demonstrated to the farmer's group in HP

Basic product	Details or variations
Dhal	• *Dhal mori* (*dhal* pressure cooked and prepared normally) • *Dhal khati* (Sour) *(dhal* pressure cooked and prepared with the addition of ingredients green mango powder*)*
Nuggets	• Ricebean *dhal* nuggets (*dhal* soaked, ground and nuggets prepared) • Ricebean *dhal* blended with black gram *dhal* (*dhal* soaked, ground and nuggets prepared) • Nuggets with ash gourd (*dhal* soaked, ground mixed with grated ash gourd and nuggets prepared) • Nuggets with papaya (*dhal* soaked, ground mixed with grated papaya)
Kandals	• *Colocasia* stem nuggets (*dhal*) soaked, ground, mixed with other ingredients, spread on *colocasia* stems and soaked.
Patorora (*Colocasia* leaves)	• Paste of ricebean *dhal* spread between layers of leaves of colocasia and rolled, then steamed and deep fried
Stuffed *Roti* (*Bhatura*)	• Ricebean *dhal* paste stuffed in fermented dough roll and deep fried
Namkeen	• Soaked ricebean *dhal* for 16 hours then sun dried for 2 hours and roasted and fried
Pakoras	• Onion *pakoras* • Plane *pakoras* • *Bhalle*
Sprouted *chat* ricebean	• Sprouted *dhal chat* with common salt • Sprouted *dhal* with onion, tomato and lemon • Sprouted *dhal* sautéed with other green vegetables
Uppuma	• Ground ricebean *dhal* mixed with other ingredients like semolina and *besan* (chickpea flour)
Siddu	• Fermented dough balls stuffed with ricebean *dhal* paste and steamed

In October and November 2007, products of ricebean with value addition were prepared (Table 2.5.2) and training sessions organized in the Trilokpur area at Mangaltika, Nayangla, Kholi, Trilokpur, and in the Khundian area at Ramnagar, Gharana, Thill and Dhenai. In all about 450 farmers, included females and males took part. The training sessions included nutritional advice on the importance of including pulses and vegetables in the diet, highlighted the importance of and method of consumption of ricebean for better nutrition, and demonstrated methods for preparation of value added products of ricebean, using locally available *Dhal* and comparing it with products produced simply using readily available utensils.

Of the ten basic products noted above, there was keen interest shown in nine: *dhal*, nuggets, *kandals*, stuffed *roti*, *namkeen*, *pakoras* and sprouted *chat*, so supporting the view that there is a potential market for value-added products from ricebean. Further organoleptic evaluations will be carried out over the coming year.

Table 2.6: Deliverables list for the period

List all deliverables, giving date of submission and any proposed revision to plans.

Del. no.	Deliverable name	Workpackage no.	Date due	Actual/Forecast delivery date	Estimated indicative person-months *)	Used indicative person-months *)	Lead contractor
7.1	Dissemination strategy established and reviewed quarterly	7	15, 18, 21	15, 18, 21	4.5	3.0	CAZS-NR
7.5	Press articles and broadcasts	7	18	18 and ongoing	2	1.67	CAZS-NR
7.6	Demonstrations "on-farm"	7	18	18 and ongoing	5	3.5	CAZS-NR
2.1	National distributions of ricebean	2	12	Draft submitted May 2007 (Final June 2007)	24	24	LI-BIRD
5.1	Diet and food preparation documented	5	10	24	30	24	UB
7.3	Project brochures	7	11	15 and ongoing	3	2	CAZS-NR
7.4	Technical documentation	7	11	18 and ongoing	3	0	CAZS-NR
7.7	Outputs on CD-ROM and/or video	7	11	18	3	1	CAZS-NR
5.2	Nutrient content analysed	5	11	30			UB
3.1	Polymorphic markers	3	18	24. Draft submitted with this report	45	45	NARC
2.2	Analysis of local knowledge	2	24	24. Draft submitted for editing	45	45	LI-BIRD

***) if available**

Table 2.7: Milestones List

List all milestones, giving date of achievement and any proposed revision to plans.

Milestone no.	Milestone name	Workpackage no.	Date due	Actual/Forecast delivery date	Lead contractor
2	Documentation of national distribution of ricebean	WP2	3	15	LI-BIRD
4	Field survey, India and Nepal	WP5	4	18	UB
5	Dissemination strategy	WP7	6	Ongoing	CAZS-NR
6	Website	WP7	6	ongoing	CAZS-NR
7	Literature review, supply chain	WP1	7	12	CAU
8	Ricebean diversity surveys	WP2	10	12	LI-BIRD
9	Report on dietary patterns and nutrition	WP5	10	26	UB
10	Identification of polymorphic markers	WP3	12	24	NARC
11	Annual meeting	WP6	12	18	CAZS-NR / all
12	Completion of second year of mother and baby trials	WP4	24	24	GVT
13	Membership of networks	WP6	12	Ongoing	All
14	2nd diet and nutrition report	WP5	24	30	UB
15	Lab analyses of nutrient contents	WP5	17	17	UB
16	Complete supply-chain field work	WP1	18	18	CAU
17	Complete field work for agro-morphological characterisation	WP2	18, 24	18, 24	LI-BIRD
18	Germplasm evaluation with markers	WP3	24	36	NARC

Section 3 – Consortium management

3.1 Consortium management tasks and their achievement; problems which have occurred and how they were solved

Management tasks for this period were as follows:

WP6: Coordination, management, integration and synthesis

These remained basically the same as for the previous year. They were to:

To maintain internal procedures for the project. The various internal procedures finalized at the initial workshop held in Kathmandu were kept under review over year 2.

To hold the 2nd Annual Meeting. This was held at the Wild Grass Resort, Kaziranga, Assam, India in October 2007. The minutes were submitted with the first interim report in November 2007.

To elaborate the detailed workplan. This was adjusted again at the 2nd Annual Meeting, and the updated barchart is attached. Experimental protocols were reviewed and updated if necessary at Technical Meetings held in Palampur in May 2007, in Bhopal in early 2008 and at a field tour in Nepal in November 2007.

To ensure partners joined relevant S Asian and other networks of relevance. A number of networks were joined in the first year, and staff remain involved in electronic discussions and on mailing lists. Staff participated in the Agrofolio electronic conference, and established links with that project (http://www.agrofolio.eu), which looks to maintain agricultural diversity and to improve the agricultural portfolio, by supporting the use of neglected and underutilised species in Asian countries. Other networks include the Global Forum on Underutilized Crops, the European and International Associations of Agricultural Economists and a number of German organisations including the Arbeitsgemeinschaft für Tropische und Subtropische Agrarforschung (Council for Tropical and Subtropical Agricultural Research). Staff also take part in various electronic for a on underutilised crops. Project deliverables are submitted to the website of the GFU as they are produced for wider dissemination.

To maintain financial reporting and monitoring procedures. The various procedures discussed and finalized at the initial project workshop have been adhered to, and further assistance has been given to partners since then with regard to completion of the various reporting formats.

To provide assistance to partners with travel arrangements. Assistance has been provided to the Asian partners with their travel arrangements both for internal travel (within India) and internationally (India – Nepal, and Nepal – India). This has included invitation letters for visas, as well as requests to institutional administrations to allow staff to travel – obtaining permission to be "off-station" is often a problem for even senior staff in the Asian institutions.

To maintain quality control. This was set up at the initial workshop, and consists of one senior staff member, not involved in the project, from each participating institution.

WP7: Output and dissemination / knowledge management

To develop the dissemination strategy. This was drafted during the project preparation phase, refined during the first year of the project, and was further refined in year 2. The Asian partners are particularly experience in disseminating outputs, and are playing a major role in these activities.

The findings from the participatory evaluation of ricebean germplasm in India and Nepal are being summarized in local languages and disseminated to communities through the most effective media, for example FM Radio networks (particularly in Nepal), TV, local papers and magazines. The scientific findings from these studies will be published in refereed journals and also posted in the project web site and linked with the web site of under utilized crops networks. They will be further disseminated through networks in India and Nepal, and efforts will be made to strengthen the effectiveness of these networks by creating linkages between the networks of both the countries.

To produce the project website. The domain name http://www.ricebean.org was registered as soon as the project was approved, and the website went live in December 2006. It has so far received over 6000 hits (verified by http://www.statcounter.com). Content so far includes staff and institutional profiles, a description of the project, and detailed information about ricebean cultivation, including a comprehensive and regularly updated bibliography of work on ricebean and related species (in particular other *Vigna* spp. and *Phaseolus vulgaris*). This includes links to either abstracts or to full papers where copyright restrictions permit. Work in still in progress to provide a secure area for to allow project staff to exchange documents and other material that needs to remain confidential – this is delayed owing to technical problems. This will replace the ricebean blog (www.ricebean.blogspot.com) which has a number of limitations with regard to the type of files that can be sent as attachments.

Other dissemination activities are noted in Annexe 1 to avoid repetition.

WP8: Monitoring and review

The tasks for this WP were to ensure physical progress in accordance with schedule and budget; assess the preliminary response by stakeholders to project activities; reporting to the coordinator and pre-meeting briefings. This has continued in a satisfactory fashion over the year. Monitoring of the progress of the field sites took place in 3 locations in India, and in Nepal.

3.3 Coordination activities

We have contacted scientists working on other underutilized crops, and gave an oral presentation for an International Conference held in the UK in September 2007. Project staff have continue to be active in a number of relevant networks, including the Global Forum on underutilized crops, and contacts in India with the All India Coordinated Research Project on Underutilised Crops of the Indian Council of Agricultural Research have been strengthened. In Nepal, linkages have been maintained with several NGOs working in the region, for example the SUPPORT Foundation (a local NGO in the far-western part of Nepal) and Rapti Agriculture Graduates Society (RAS)-Nepal (a local network of agricultural graduates in the mid-western region of Nepal), as well as with the International Centre for Integrated Mountain development (ICIMOD). Other working relationships have been established with farmers' groups and government Agricultural Development Offices in Nepal.

Table 3.3.1. Details of meetings held in year 2

Date	Location	Attendance	Purpose	Management outcomes*
May 2 – 4, 2007	AAU, Jorhat, India	Dr JP Yadavendra (GVT, Dahod); Dr KD Joshi (CAZS-NR, Kathmandu); Dr N Kumar (CSHPKV, Palampur); Dr SB Neog, Ms R Bora, Mr.R Mili (AAU, Jorhat)	Finalisation of work on farmer preferences; Finalise field protocols; planning for annual workshop;	Field protocols finalized; workshop arrangements in hand;
October 7 – 11, 2007	Kaziranga, Assam, India	Dr PA Hollington (Coordinator), Prof JR Witcombe, Dr D Harris, Dr KD Joshi (CAZS NR); Prof RAE Mueller, Ms D Bürgelt (CAU); Dr P Andersen (UB); Dr S B Neog, Miss R Bora, Mr S Timung (AAU Jorhat); Dr	2nd annual meeting. Administrative and budget reports for year 1; Overview of scientific progress, Status of deliverables, Detailed scientific reports by WP, Dissemination and	Partners agreed to contribute additional website pages. Agreed to continue working to obtain permission for germplasm exchange from India To look at the

		JP Yadavendra, Dr LK Sharma (GVT Dahod); Mr R. Dwiwedi (GVT Jhabua); Dr N Kumar, Mr S Kumar, Mr V Sharma (CSJHPKV, Palampur); Mr R Gautam, Mr KP Devkota, Mr A Khanal (LI-BIRD, Pokhara); Dr J Bajracharya, Mr RC Prasad (NARC Kathmandu)	outreach activities, Plan for disseminating knowledge; Plans for next reporting period; Issues directly affecting farmers and industry; Analysis of Mother and baby trials, data formats and yield units; field visits to ricebean growing areas	possibility of grey literature publication through ICIMOD; To provide templates for posters and brochures to the other partners; To pursue additional members for the Ricebean Network; To cost production of a CD-ROM or video, and produce for distribution in Nepal and India
22 – 28 October, 2007	Himachal Pradesh and MP, India	Dr JP Yadavendra, Dr N Kumar, Dr SB Neog	Monitoring and evaluation of field sites in India	*Scientific only*
2 – 7 November, 2007	Chitwan, Kavre and Ramechap, Nepal	Dr J Bajracharya (NARC), Dr SP Srivastava (NGLRP Coordinator), Dr RC Prasad (ARS Kavre Coordinator), Mr K Devkota, Mr A Khanal (LI-BIRD), Mr I Poutel (IAAS Rampur)	Monitoring and evaluation of field sites in Nepal	*Scientific only*
April 24-25, 2008	Bhopal	Dr JP Yadavendra (GVT); Dr N Kumar (CSKHPKV); Dr SB Neog (AAU)	Annual technical programme meeting of the Indian partners	*Scientific only*

*Scientific outcomes of the meetings are noted in the respective section of the progress report.

Partners have been in almost continuous communication by email and telephone, as well as face to face meetings, and the CAZS Regional Coordinator has visited all the Asian partners at least once. In addition to the Annual Meeting in October (minutes attached with the First Interim Report) several other meetings have taken place (Table 3.3.1).

Annex 1 – Plan for using and disseminating the knowledge

Section 1 - Exploitable knowledge and its use

As noted in both the project proposal and confirmed in Annexe 1, this project involves the participation of a large number of stakeholders, most of whom are small resource-poor farmers. So far as intellectual property is concerned, the property rights and other ethical issues concerned with Participatory Technology Development are far behind technical advances. However, we are of the firm opinion that that joint collaboration should mean joint benefit sharing. The major outputs of the project, improved technologies (which includes germplasm), are public goods and will be placed in the public domain, so the question of exploitable results for industrial or commercial exploitation does not in the main arise. However, great care will be taken when disseminating germplasm to protect the IPR of the project partners, and all organisations requesting germplasm or DNA will be required to sign a materials transfer agreement.

However, this does not mean we are unaware of the potential for commercial exploitation, although this is unlikely for ricebean as a protein crop targeted at the poorest sectors of society in India and Nepal. If improved germplasm is developed it may well attract the interest of seed companies, and if this is the case it will be licensed to them on a basis that ensures the interests of both the partners and the participating stakeholders are maintained and protected.

The two economic results that are capable of exploitation in any sense are the MLTVI, and the knowledge of the supply chain for ricebean and other legumes in Nepal and India. These will be developed by Kiel, working with the Asian partners. However, nothing that comes out of the economics research is commercially exploitable because IPRs cannot be assigned to economics data, ideas, concepts, results of hypothesis testing, and insights. Such things are not embodied in some technique and cannot be patented, even in principle. Without IPR there is nothing to sell and therefore these results are of no commercial value. In a sense, the economics research is purely for benefit of the public at large.

However, the knowledge produced will clearly be of benefit to various groups as mentioned in the table. Market systems analysis in both India and Nepal will be helpful if fully exploiting the market potential of the crop. The partners working on this work package will need to closely work with the partners working for market analysis of ricebean.

Promising ricebean landraces and the better understanding about the nutritional value of ricebean will form the basis for future research. For example, most promising landraces can be utilized as parents for ricebean improvement. Similarly new knowledge generated from nutrient analysis will be widely disseminated using various media promoting the use of ricebean largely. This will also create new market demand for the crop ultimately contributing to greater use and conservation of ricebean. This material will be developed by the partners working with farmers. It is our intention that it should be in the public domain, although subject to materials transfer agreements in order to protect the interests of the farmers in particular. Wider adoption of farmer preferred and high yielding ricebean landraces will lead to substantial increase in the yield of ricebean, contributing to increase in income, food and nutritional security of the farmers. The wider adoption of ricebean, and work to improve it, will be assisted by two of the early deliverables of the project.

Publication in India and Nepal of Deliverable 3.1 on the national distributions of ricebean will benefit researchers and the agricultural sector generally by providing information on the geographical distribution and pocket areas of the crop. It is hoped that at some stage we will also be able to incorporate this information into a GIS using GPS. Similarly, publication of the local knowledge will provide information on use and diversity of crop, farmer-preferred traits, problems associated with ricebean and so on, to allow the incorporation of this with scientific knowledge and so contribute to crop improvement.

The molecular material, the responsibility of CAZS-NR and NARC, is somewhat complicated as markers will be developed using existing material obtained under an MTA, Primer sequences and markers will be placed in the public domain, and any tests for distinguishing varieties will be available under a licence, subject to the necessary agreement of these other parties.

The core of the nutrition WP is to establish better knowledge of a variety of South Asian diets, and the role of pulses and in particular ricebean in that. These data are presently largely unknown and will fall into a basic research category but can be crucial for further research on nutrition intervention and impact analysis of agrarian change. UB will take responsibility for this aspect of the project, but again the data will be in the public domain.

Section 2 – Dissemination of knowledge

On-farm germplasm evaluation trials were visited by project staff at the tours held before the initial workshop, and after the second annual workshop, and a number of farmers their families participated in the event, discussing ricebean with the project staff. In 2007 project staff attended two conferences, one in the UK in September, and one in India. These brought the project activities to a wide audience. A number of the scheduled dissemination activities for the first year were postponed as a result of the late start to the project activities but have begun in year 2. We still intend to try to develop a mechanism of mentoring with the participating communities after the completion of the project, and if possible to conduct a separate impact assessment five years post-project. This will be carried out by LI-BIRD in Nepal, and funds will be sought to allow the same to take place in India. We remain strongly of the opinion that outcomes should be subject to intensive upscaling efforts both during and after the project.

Website

The website http://www.ricebean.org has received over 6000 hits (as at 10 May 2008). Content so far includes staff and institutional profiles, project reports, presentations and deliverables, a project description, background information about ricebean, a page on the nutritional aspects, and a comprehensive bibliography on ricebean and related species (in particular other *Vigna* spp. and *Phaseolus vulgaris*), as well as links to either abstracts or to full papers where copyright restrictions permit.

Networking activities

Contacts have been made with the All-India Coordinated Research Project on Underutilised Crops of the Indian Council of Agricultural Research, and Dr Yadavendra was invited to give a presentation on FOSRIN to the All India Coordinated Research Network on Underutilized Crops in New Deli, May 2008. This will be fully reported in the next report. Dr Naveen has provided 25 demonstrations of promising ricebean lines in different areas of Himachal Pradesh. In Nepal, linkages have been made with several NGOs: the SUPPORT Foundation (a local NGO in the far-west), and the Rapti Agriculture Graduates Society (RAS)-Nepal (a local network of agricultural graduates in the mid-western region of Nepal), as well as with the International Centre for Integrated Mountain development (ICIMOD). We developed other relationships with farmers' groups and government Agricultural Development Offices in Nepal.

Project staff participated in various electronic discussions and mailing lists, for example the Agrofolio electronic conference, and established links with that project (http://www.agrofolio.eu). Other networks joined include the Global Forum on Underutilized Crops, the European and International Associations of Agricultural Economists and various German organizations. Dr Hollington joined the Mountain Forum (MF)/Asia Pacific Mountain Network (APMN) and the FAO email discussion forum on underutilised crops. All project deliverables, and a summary of the work on ricebean, are posted at the GFU website http://www.underutilized-species.org/default.asp

Farmer and community activities

A number of focus group discussions and farm field days on ricebean at different places of project area in India were conducted during the crop season and after harvesting of the crop, and 20-40 farmers attended each of these programmes. Three field days were organised by GVT, and two each by CSKHPKV and AAU, while GVT organised three, and CSKHPKV two, focus group discussions. AAU held an exhibition in which ricebean was featured, while a number of demonstrations with farmers to show ricebean growing as a mixed crop with maize were held, including 52 in Himachal Pradesh. In Nepal, the various mother and baby trials are acting as demonstrations, and in India GVT had carried out a large-scale demonstration and seed production trial. Stakeholder groups, including farmers, government staff, etc., did participatory monitoring of all project sites in India and Nepal.

Exhibitions

In Nepal, LI-BIRD participated in the All-Nepal Farmers Conference, with a stand to show ricebean samples and highlight the activities of the project. Over 10,000 farmers from throughout the country attended. LI-BIRD have also maintained a ricebean exhibition in the conference hall at their headquarters in Pokhara.

Mr AR Khanal from LI-BIRD participated in and displayed ricebean samples and nutritional information, and organized a display of ricebean recipes in the Agro fair held from 8-11 February 2008 in Butwal, Nepal. The Fair was inaugurated by the Honourable Minister for Agriculture and Cooperatives, Mr. Chhabilal Bishwakarma, and a total of 45 Agro Enterprises, organizations and farmers' groups exhibited. The fair was attended by around 45,000 visitors. Twenty diverse ricebean samples collected from various parts of Nepal with differing seed size and colour were displayed along with relevant information (Figure A1-1). Visitors were surprised by the diversity of the ricebean seeds, and requested information on the nutritional value: some also asked for seeds of their preferred varieties.

A flex-printed poster (1200 x 1800 mm) entitled "*Bahupayogi Jhilunge Bali: Samrachhan ra Prabardhan Garaun*." (Let's conserve and promote our traditional multipurpose crop ricebean) was displayed (Figure A1-1) in the fair. Information and importance of ricebean crop were mentioned in the poster.

Figure A1-1: Display of ricebean samples in the fair (left) and flex-printed poster (right)

Figure A1-2: Food fair organized by LI-BIRD during agro-fair (left) and *Batuk*, a popular recipe prepared from ricebean (right).

On 10th February 2008, a food fair for underutilized species was organised in collaboration with *Bhuneshwor Gharbagaicha mahila Krishak Samuha* (Women farmers' group of Rupandehi). Recipes were displayed with preparation techniques and information on the crop (Figure A1-2), recipes including *Batuk*, *Biramala,* and local recipes of the Tharu ethnic communities such as *Dhikari*, *Anadi ko Bhuja* were offered to the visitors and their responses collected.

Visitors were interested in the diversity of ricebean recipes available, and the display of ricebean information and its nutritional value made them aware of the hidden treasure of this traditional crop. Most visitors requested more information on ricebean. The Fair seemed to be supportive in two way communication for sharing experiences about ricebean between researchers and farmers (Figure A1-3). Beside, we prepared different recipes from ricebean and offered to visitors to taste them. The Food Fair showing different recipes not only made rural visitors inquisitive, but was equally helpful to spread ricebean more than locally popular in urban areas, and some visitors asked for seeds of their preferred landraces.

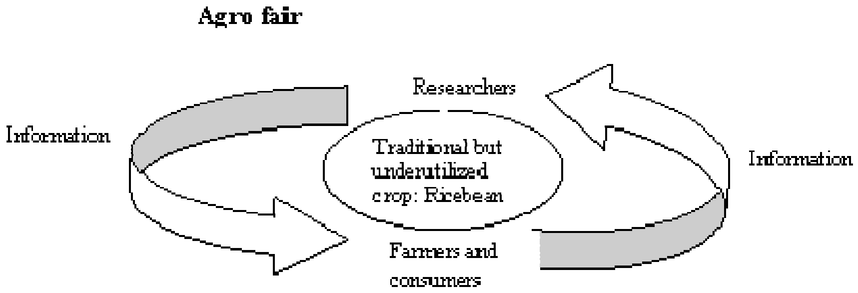


Figure A1-3. Model for information sharing between researchers and farmers through agro-fair.

Publications

Although no journal articles have yet been produced the project has produced a number of scientific publications to date:

1) Mueller, R.A.E., Buergelt, D. and Seidel-Lass, L. (2007): *Supply Chains and Social Network Analysis.* Paper presented at the 1st International European Forum on Innovation and System Dynamics in Food Networks, 15.-17.2.2007, Innsbruck-Igls, Austria.

2) Joshi, K.D., Bhandari, B., Gautam, R., Bajracharya, J. and Hollington, P.A. (2007) *Ricebean: a multipurpose underutilised legume*. Paper presented at the 5th International Symposium on Underutilised crops, Southampton University, UK, September 3-4, 2007.

3) Gautam, R., Kumar, N., Yadavendra, J.P., Neog, S.B., Thakur, S., Khanal, A., Bhandari, B. and Hollington, P.A. (2007) *Distribution of ricebean in India and Nepal.* Food Security through Ricebean Research in India and Nepal (FOSRIN) Report 1. Pokhara, Nepal, Local Initiatives for Biodiversity, Research and Development and Bangor, Wales, UK, CAZS Natural Resources, College of Natural Sciences, Bangor University, 30 pp.

4) Bajracharya, J., Singh, S., Dangol, B., Hollington, P.A. and Witcombe, J.R. (2008) Identification of polymorphic markers in ricebean (*Vigna umbellata*) Food Security through Ricebean Research in India and Nepal (FOSRIN) Report 2. Kathmandu, Nepal, Nepal Agriculture Research Council and Bangor, Wales, UK, CAZS Natural Resources, College of Natural Sciences, Bangor University, 20 pp

Other publications

5) Hollington, P.A. (2007) Ricebean Network: research to promote the adoption of “orphan” crops to improve food security in marginal areas. *The House Magazine*, June 2007, pp 54-55.

6) Kumar, N., Bhandari, J.C., Thakur, S.J. and Chahota, R. (2007) *Successful production of rice bean in north-western Himalaya*. Fodder Production and Grassland Management Centre, CSKHP Agriculture University, Palampur, India. 6 pp. (Also available in Hindi)

A number of other publications have also been produced (brochures were attached to the First Interim Report):

- Popular article published in Assamese Daily.
- Leaflets in three languages (Assamese, English and *Karbi*)
- Bulletin published in Assamese and English.
- A brochure also made, containing the ricebean cultivation package and practices which may be useful to ricebean growers and also help to disseminate of our project activities in the target area by GVT

- Two pamphlets published from Palampur on ricebean cultivation
- An article (submitted with the First Interim Report) has appeared in the UK Parliamentary magazine, “The House” with wide circulation among opinion formers.
- LI-BIRD produced and distributed a project information brochure in Nepal, and uploaded project information to their website
- In HP, Dr Naveen has produced extension booklets in English and in Hindi covering ricebean, as well as a popular article in Hindi.
- A general project brochure has been produced in Bangor, and templates circulated to partners for more specific WP-related ones.
- We have produced a template for a FOSRIN poster.

A project desk calendar was also brought out by GVT for the dissemination of our project activities among the stakeholders and farmers. LI-BIRD also highlighted the project in their calendar.

Awareness-raising activities

In October and November 2007, Dr Kumar organised awareness-raising sessions in eight villages in two districts of HP. Around 450 farmers and villagers (both males and females) took part, and received nutritional advice on the importance of pulses and vegetables in the diet, and were shown different methods of preparation for ricebean. Out of ten products or preparations offered, there was interest in seven.

In May 2007, Dr Bajracharya was invited to attend a training workshop on species diversity mapping under the Western *Terai* Landscape Conservation programme.

Press, TV and radio

Dr Bajracharya participated in the Nepalese TV Agricultural News Programme on November 23, 2007 concerning FOSRIN and the activities going at Khumaltar and other research sites, as well as a brief introduction of the crop itself.

Finally, she also gave a talk to scientific and technical staff in the ABD, NARC about FOSRIN and its on-going activities in NARC and other partner institutions.

We intend next year to produce a video documentary in both English and Nepali to summarize the project findings, farmers’ practices, knowledge and information on ricebean, for the benefit of researchers, farmers and government offices.

Annexe 2: Protocol for on-farm/on-station agro-morphological trials in 2007

1. **Evaluation of core collection under mixed/sole cropping system** (Selected accessions of LI-BIRD and NARC)

Rationale and brief introduction

Ricebean is a minor leguminous crop in Nepal identified by the FOSRIN project with the values in food security for poor rural people in Nepal and India. Since maize is the staple food crop for the hills, mixed cropping of ricebean with summer maize is very common practice in many hilly areas of the country. Ricebean landraces are under cultivation in and across the districts of the country under varied agroclimatic conditions, varied soil conditions for their cultural, religious and many use values as fodder and food. The characterization studies using different marker systems have shown the existence of great diversity in traditional varieties of different crops at phenotypic and genetic levels. This diversity is of immense value which permits a landrace to adapt to changes in environment, cultural practices and biotic and abiotic stresses and determines the evolutionary potential of a species. Information on the level and distribution of genetic variation within a species can be obtained empirically from differences in agro-morphological and physiological traits, biochemical traits and DNA sequences. Agro morphological traits and their characterization is the first and basic method used in measuring the extent of diversity. Polygenic traits (quantitative) and major gene traits (qualitative) are intensively used to quantify genetic diversity of crop species with low to high proximity towards DNA diversity. This is therefore a part of activity to assess the ricebean landrace diversity of Nepal in the FOSRIN project.

Ricebean germplasm collected in the past and in 2006 have been evaluated for agro-morphological variation. There was a great variation between accessions for several qualitative and some of the quantitative traits, e.g. yield and yield components, crop duration and growth habit. Some of these accessions with diversity in local names and collected from different parts of the country have also been studied for their polymorphisms for some of the major traits using simple sequence repeat SSR) markers. A meeting held in March in Kathmandu agreed that a selective number of ricebean accessions from NARC and LIBIRD collections would categorized as core collection and remaining accessions will be included into non-core collection. Core collection currently consists of 35 accessions identified by NARC and 15 accessions will be identified from the collection of LI-BIRD. These 50 accessions will be considered as core collections. These accessions were collected and evaluated in 2006 crop season in Khumaltar research station and Gulmi on-farm. Evaluation of ricebean accessions will be done at four sites; Gulmi, Khumaltar, Kavre and Rampur. All the core collections will be evaluated at each of these sites along with additional 50 accessions each from non-core collections.

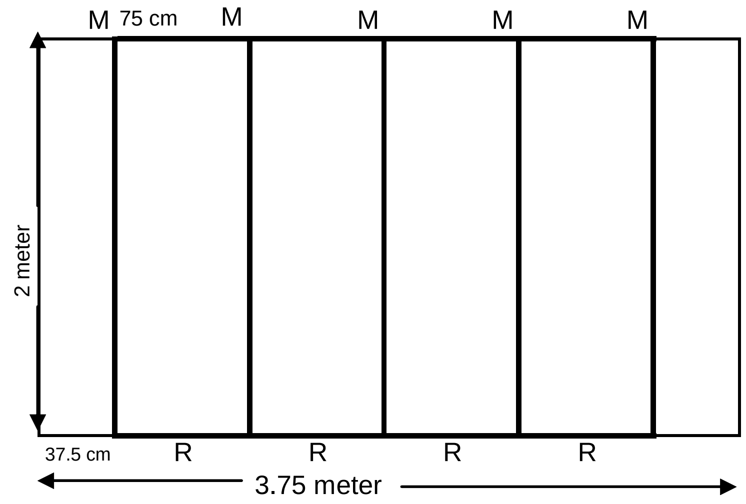
Objectives:

- To characterize the ricebean landraces for agro-morphological traits
- To measure the extent and amount of diversity for observed agro-morphological traits
- To identify the ricebean genotypes for further enhancement and improvement

Output: Extent of morphological and genetic diversity and their geographic distribution will be determined in ricebean and few superior genotypes with desired traits will be identified. The performance of selected accessions will be assessed under maize intercropping and sole cropping systems. The trial in Gulmi will be done under inter-cropping conditions, while in other three sites it would be under sole cropping. The materials and process/methods/steps will be carried as per the details described below:

Particulars	**Trial details**
Number of accessions	35 NARC + 15 LI-BIRD = 50 accessions (List of accessions under core collection is attached along with Proforma recording data sheet)
Number of trials	One trial at each testing site - Gulmi, Khumaltar (ABD), Kavre (HCRP) and Rampur (NGLRP) (In Gulmi it will be conducted on-farm under inter-cropping system with maize while in other sites it will be under sole crop system)
Trial design	RCBD with two replications
Plot size (4 rows per plot)	7.5 m^2 (2 x 3.75) per accession
Spacing	Maize crop: 75 cm x 25 cm (5 rows of 2m length) Ricebean: 75 cm x 50 cm (4 rows of 2 m length)
Land preparation, manures and fertilizers	As per farmers practice. (record the quantity of FYM or compost used). Do not apply chemical fertilizers
Time and method of planting	<u>For inter-cropping with maize:</u> Normal time of planting: third week of April (Baisakh 2nd week) for maize and plant ricebean after one month of planting maize Plant maize and ricebean in alternate rows in the third week of April (Baisakh 2nd week) BUT it would depend on the rainfall Dibbling of ricebean in between maize rows after first digging (30 days after maize seeding) in a spacing of 75 x 50 cm <u>For sole cropping:</u> Plant ricebean on raised seed beds in 3rd week of May depending upon the monsoon rainfall and after establishment earthen up more to raise the seed bed with proper drainage channels
Variety of maize used	Any improved variety adapted in the area
Plant Population per plot	40 maize plants and 16 ricebean plants
Seed rate	For both the crops, plant two seeds per hill but retain only single plant after full establishment
Intercultural operations	One weeding of maize before ricebean seeding as per farmers practice One weeding for ricebean at the time of maize earthing up No need for any other intercultural operations Staking provided for accessions with indeterminate growth habit
Harvesting, threshing, storage	As per the farmers practice. Avoid any chance of varietal mixtures during harvesting, threshing and storage. Sun dry seeds thoroughly and store in air tight containers, e.g. plastic bottles. Use naphthalene balls to avoid insect damage
Experimental data collection, computation and analysis	The observed data will be compiled and multivariate analysis will be carried and the results on agromorphological diversity will be compared with genetic diversity at DNA level using SSR markers.

Note: Farmer is encouraged to grow ricebean by him/herself under maize intercropping system near by the trial plots as per his own practice and varieties/landraces. This will offer us to obtain information regarding farmers practice and also some data for better comparison.



Note: M=Maize row and R=Ricebean row

Figure 1. Field lay-out plan for ricebean trial with maize intercropping/sole cropping

Agro-morphological traits to be recorded:

1) Date of planting, flowering and maturity
2) Plant height at maturity (Average of five plants)
3) Growth habit
4) Flower colour
5) Pod length: Five pods each from lower, Medium and upper strata of sample plant)
6) Number of pods per plant: (Average of five randomly sampled plants)
7) Number of seeds per pod: (Five pods each from lower, Medium and upper strata of sample plant)
8) Seed colour
9) Total grain yield per plant: (Average of five sample plants)
10) 100 seed weight

Note: *Please follow the states of traits at the stated stage of the plant. General information of the study, Ricebean descriptor and the observation sheet are attached in Proforma for conducting the experiment and recording the data.*

2. Evaluation of non-core collections: Sole planting

All other accessions excluding the accessions under core collection will be evaluated separately under sole cropping. As in last year, this will be a non-replicated observation nursery with two rows of 2.5 m. Most the accessions under preliminary study observed with indeterminate in growth habit with luxurious vegetative growth. Therefore, more distance between two plots is needed for easy movement between the accessions for taking measurement or for any intercultural operations and also for not inter mixing of vines of accessions of two plots. The details protocol for evaluation of these accessions is as follows:

Particulars	**Trial details**
Number of accessions	50 at each site (This set will be tried to replicate in two sites at the least)
Design and plot size	Non-replicated observation nursery 3 rows of 2.5 m length (15 plants/plot)
Number of trials	One in each location (Gulmi, Khumaltar, Kavre and Rampur)
Land preparation	As per farmers practice
Manures and fertilizers	As per the farmers' practice (record the quantity of FYM or compost used). No need to apply chemical fertilizers.
Number of seeds per hill	Plant three seeds per hill but retain only single plant after full establishment Plant on raised bed to avoid any loss of plants due to water logging
Spacing	Row to row - 1 m Plant to plant - 50 cm Plot to plot - 1 m
Intercultural operations	Keep the crop free from the weeds. Provide stakes for the indeterminate landraces. No need for any other intercultural operations
Harvesting, threshing, storage	As per the farmers' practice. Avoid any chance of varietal mixtures during harvesting, threshing and storage. Sun dry seeds thoroughly and store in air tight containers, e.g. plastic bottles. Use naphthalene balls to avoid insect damage
Experimental data collection, computation and analysis	The observed data will be compiled and multivariate analysis will be carried and the results on agromorphological diversity will be compared with genetic diversity at DNA level using SSR markers.

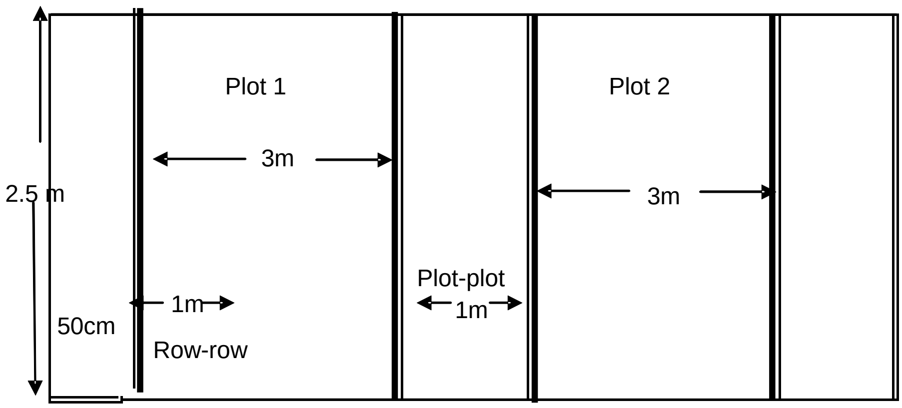


***Figure* 3.** Field lay-out plan for Ricebean non-core accessions (Sole planting)

Agro-morphological traits to be recorded**:** Same as in core evaluation and follow the provided descriptor.

3. Evaluation of better performing accessions in mother trials under maize intercropping system in Gulmi

Particulars	**Mixed planting with maize**
Number of accessions	8 entries (selected mid and late maturing)
No. of trials	10 (5 at Darbar and 5 at Simichaur) Can we not increase the number of mother trials?
All other details including plot size and design	Same as for the core collection accessions evaluated under maize intercropping system

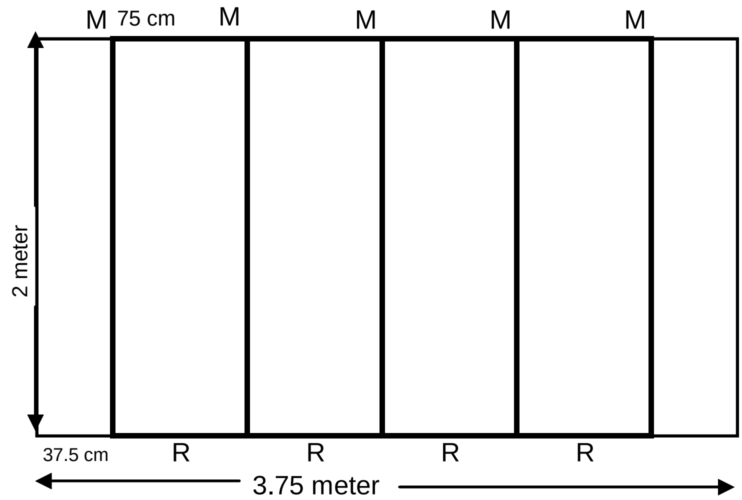


Figure 4: Field lay-out plan for Ricebean mother trial (intercropping with maize)

<u>Agro-morphological traits to be recorded:</u>

1) Date of planting, flowering and maturity
2) Plant height at maturity (Average of five plants)
3) Growth habit (determinate/Semi-determinate/Indeterminate)
4) Flower colour
5) Pod length
6) Number of pods per plant: (Average of five sample plants)
7) Number of seeds per pod: (Five pods from lower, Medium and upper strata of each plant)
8) Seed colour
9) Total grain yield per plant in g: (Average of five sample plants)
10) 100 seed weight in g at uniform moisture content:

Ranking of ricebean landraces in mother trials

(Ranks: 1=Worst and 8= Best)

Date:………………..

Farmers Name: Location:

Total No. of participants in FGD: Male: Female:

Important traits	**Ricebean landraces**								
	1	2	3	4	5	6	7	8	
Pod length									
Non-seed bearing pods									
Grain size									
Maturity									
Yield potential									
Fodder yield									
Grain colour									
Grain type									
Disease tolerance									
Insect tolerance									
Others									
Others									
Overall preference									
Average score									

Data- sheet

Name of collaborating farmer:

VDC: Ward: Village:

Altitude: **Aspect:** **Plot size:**

Soil fertility status: a) High b) Medium c) Low

Date of planting: **Date of harvesting:**

SN	Accession	Date of emergence	Date of 50% flowering	Plant height at maturity (cm)	Pod length (cm)	Days to maturity	Plant stand at harvest (no.)	Growth habit	Flower colour	No. of pods/ plant	No of seeds/pod	Seed colour	Disease (1-5)	Insect (1-5)	Field weight (kg)	Moi cont (%)

Note: †=In overall ranking highest number for most preferred landrace

Annexe 3. Details of the germplasm accessions used in Nepal in 2007

Table A3-1: Core accessions for on-farm (Gulmi) and on-station (Khumaltar, Kabre and Rampur) agro-morphological characterization of ricebean, 2007

S No	Accession	Districts collected	Local name	Collecting organisation
1	NPGR-00007	Nuwakot	*Chhirbire masyang*	ABD§
2	NPGR-00008	Nuwakot	*Panhelo masyang*	ABD
3	NPGR-00010	Lalitpur	*Masyang*	ABD
4	NPGR-00012	Nuwakot	*Rato masyang*	ABD
5	NPGR-00015	Bhaktapur	*Masyang*	ABD
6	NPGR-00073	Gulmi	*Thulo panhelo masyang*	ABD
7	NPGR-00076	Arghakhanchi	*Dhawanse masyang*	ABD
8	NPGR-00087	Pyuthan	*Masyang*	ABD
9	NPGR-00090	Dang	*Jhilinge masyang*	ABD
10	NPGR-00194	Kabre	*Masyang*	ABD
11	NPGR-01975	Baitadi	*Baramase masyang*	ABD
12	NPGR-05364	Bhojpur	*Masyang*	ABD
13	NPGR-05368	Bhojpur	*Bhage masyang*	ABD
14	NPGR-05370	Terhathum	*Rato ghore*	ABD
15	NPGR-05373	Gorkha	*Masyang*	ABD
16	NPGR-05377	Lamjung	*Gurans*	ABD
17	NPGR-05382	Tanahu	*Masyang*	ABD
18	NPGR-05384	Mugu	*Gurans*	ABD
19	NPGR-05386	Humla	*Gurans*	ABD
20	NPGR-05391	Bajura	*Ghore mas*	ABD
21	NPGR-05396	Illam	*Banmara masyang*	ABD
22	NPGR-05420	Dhankuta	*Ghore mas*	ABD
23	NPGR-05423	Dhankuta	*Seto mas*	ABD
24	NPGR-05432	Baitadi	*Gurans*	ABD
25	NPGR-05565	Okhaldhunga	*Masyang*	ABD
26	NPGR-06591	Mugu	*Masyang*	ABD
27	NPGR-06657	Kalikot	*Rato masysng*	ABD
28	NPGR-06756	Humla	*Gurans*	ABD
29	NPGR-07583	Jhapa	*Masyang*	ABD
30	NPGR-07882	Bajhang	*Masyang*	ABD
31	NPGR-08380	Myagdi	*Syaltung*	ABD
32	NPGR-08382	Banglung	*Syaltung*	ABD
33	NPGR-09391	Syangja	*Masyang*	ABD
34	NPGR-09461	Panchthar	*Masyang*	ABD
35	NPGR-09464	Taplejung	*Masyang*	ABD
36	LRGR42	Surkhet	*Siltung*	LIBIRD
37	LRGR43	Surkhet	*Siltung*	LIBIRD
38	LRGR44	Surkhet	*Siltung*	LIBIRD
39	LRGR75	Pyuthan	*Raiyans*	LIBIRD
40	LRGR91	Dang	*Siltung*	LIBIRD
41	LRGR99	Palpa	*Jhilinge*	LIBIRD
42	LRGR101	Palpa	*Jhilinge*	LIBIRD
43	LRGR102	Palpa	*Jhilinge*	LIBIRD
44	LRGR103	Palpa	*Jhilinge*	LIBIRD
45	LRGR107	Palpa	*Jhilinge*	LIBIRD
46	LRGR111	Gulmi	*Jhilinge*	LIBIRD
47	LRGR117	Gulmi	*Jhilinge*	LIBIRD
48	LRGR129	Palpa	*Siltung*	LIBIRD
49	LRGR137	Kaski	*Masyang*	LIBIRD
50	LRGR152	Kavre	*Masyang*	LIBIRD

§ABD: Agricultural Botany Division, NARC; LI-BIRD: Local Initiatives for Biodiversity, Research and Development

Table A3-2: Non-core accessions (set 1) for on-station agro-morphological characterization of ricebean (Rampur and Khumaltar), 2007. All collected by ABD

S No	Accession	Districts collected	Local name	S No	Accession	Districts collected	Local name
1	NPGR-00006	Nuwakot	*Masyang*	26	NPGR-05401	Ilam	*Masyang*
2	NPGR-00009	Nuwakot	*Pahelo masyang*	27	NPGR-05407	Ilam	*Masyang*
3	NPGR-00011	Nuwakot	*Masyang*	28	NPGR-05408	Ilam	*Masyang*
4	NPGR-00013	Nuwakot	*Dhade kalo masyang*	29	NPGR-05409	Ilam	*Masyang*
5	NPGR-00074	Arghakhanchi	*Khaire masyang*	30	NPGR-05411	Ilam	*Masyang*
6	NPGR-00092	Dang	*Jhilinge seto masyang*	31	NPGR-05412	Dhankuta	*Masyang*
7	NPGR-00093	Pyuthan	*Chhirbire masyang*	32	NPGR-05415	Dhankuta	*Masyang*
8	NPGR-00193	Kabhre	*Masyang*	33	NPGR-05416	Dhankuta	*Masyang*
9	NPGR-00195	Kabhre	*Masyang*	34	NPGR-05417	Dhankuta	*Ghore mas*
10	NPGR-00197	Kabhre	*Masyang*	35	NPGR-05425	Dhankuta	*Masyam*
11	NPGR-00198	Kabhre	*Masyang*	36	NPGR-05429	Ilam	*Masyam*
12	NPGR-00199	Kabhre	*Masyang*	37	NPGR-05430	Baitadi	*Masyang*
13	NPGR-01972	Ilam	*Masyang*	38	NPGR-05435		*Gurans*
14	NPGR-01974	Ilam	*Seto masyang*	39	NPGR-06725	Humla	*Gurans*
15	NPGR-05367	Bhojpur	*Rato masyang*	40	NPGR-07883	Bajura	*Masyang*
16	NPGR-05371	Bhojpur	*Naga masyang*	41	FOSRIN 4	Dailekh	*Gurans (Masyang)*
17	NPGR-05376	Gorkha	*Masyang*	42	FOSRIN 5	Ilam	*Thulo Masyang*
18	NPGR-05380	Lamjung	*Masyang*	43	FOSRIN 6	Kathamndu	*Masyang*
19	NPGR-05381	Lamjung	*Thulo masyang*	44	NPGR-08381	Myagdi	*Syaltung*
20	NPGR-05383	Tanahun	*Masyang*	45	NPGR-98383	Baglung	*Syaltung*
21	NPGR-05394	Dhankuta	*Rato masyang*	46	NPGR-09462	Panchthar	*Masyang*
22	NPGR-05397		*Masyang*	47	NPGR-09466	Taplejung	*Rato masyang*
23	NPGR-05398	Ilam	*Masyang*	48	NPGR-09691	Bajura	*Gurans*
24	NPGR-05399	Ilam	*Masyang*	49	NPGR-09710	Bajhang	*Gurans*
25	NPGR-05400	Ilam	*Masyang*	50	NPGR-10476	Gulmi	*Jhilinge*

Table A3-3: Non-core accessions (set 2) for on-station agro-morphological characterization of ricebean (Kabre and Khumaltar), 2007 (All collected by ABD)

S No	Accession	Districts collected	Local name	S No	Accession	Districts collected	Local name
1	NPGR-00004	Nuwakot	*Masyang*	26	NPGR-05388	Humla	*Gurans*
2	NPGR-00005	Nuwakot	*Masyang*	27	NPGR-05392	Bajura	*Rangale mas*
3	NPGR-00014	Nuwakot	*Dhade rato masyang*	28	NPGR-05393	Dhankuta	*Seto masyang*
4	NPGR-00072	Gulmi	*Pahenlo rato masyang*	29	NPGR-05395		*Thulo rato ghore*
5	NPGR-00075	Arghakhanchi	*Dhani masyang*	30	NPGR-05402	Ilam	*Masyang*
6	NPGR-00088	Pyuthan	*Chhirbire masyang*	31	NPGR-05403	Ilam	*Masyang*
7	NPGR-00089	Dang	*Khaire masyang*	32	NPGR-05404	Ilam	*Masyang*
8	NPGR-00091	Dang	*Chhirbire masyang*	33	NPGR-05405	Ilam	*Masyang*
9	NPGR-00183	Kabhre	*Masyang*	34	NPGR-05406	Ilam	*Masyang*
10	NPGR-00184	Kabhre	*Masyang*	35	NPGR-05413	Dhankuta	*Masyang*
11	NPGR-00185	Kabhre	*Masyang*	36	NPGR-05414	Dhankuta	*Masyang*
12	NPGR-00186	Kabhre	*Masyang*	37	NPGR-05418	Dhankuta	*Ghore mas*
13	NPGR-00187	Kabhre	*Masyang*	38	NPGR-05419	Dhankuta	*Ghore*
14	NPGR-00188	Kabhre	*Masyang*	39	NPGR-05422	Dhankuta	*Masyam*
15	NPGR-00189	Kabhre	*Masyang*	40	NPGR-05424	Dhankuta	*Masyam*
16	NPGR-00190	Kabhre	*Masyang*	41	NPGR-05428		*Gurans*
17	NPGR-00191	Kabhre	*Masyang*	42	NPGR-05431		
18	NPGR-00192	Kabhre	*Masyang*	43	NPGR-05436	Baitadi	*Gurans*
19	NPGR-01973	Ilam	*Banmara masyang*	44	FOSRIN 1	Ramechhap	*Masyang*
20	NPGR-05365	Bhojpur	*Seto masyang*	45	FOSRIN 2	Dailekh	*Situng*
21	NPGR-05366	Bhojpur	*Kalo masyang*	46	FOSRIN 3	Kavre Palanchok	*Masyang*
22	NPGR-05372	Gorkha	*Masyang*	47	NPGR-09463	Panchthar	*Masyang*
23	NPGR-05374	Gorkha	*Masyang*	48	NPGR-09465	Taplejung	*Masyang*
24	NPGR-05378	Lamjung	*Gurans*	49	NPGR-09690	Bajura	*Gurans*
25	NPGR-05379	Lamjung	*Masyang*	50	NPGR-10459	Arghakhanchi	*Jhilange*

Table A3-4: Non-core accessions (set 3) for on-farm and on-station agro-morphological characterization of ricebean (Gulmi and Khumaltar), 2007 (Nos. 1, 2 and 3 unknown, all others collected by LI-BIRD)

S No	Accession	Districts collected	Local name	S No	Accession	Districts collected	Local name
1	NPGR-00196	Ilam	*Masyang*	36	LRGR-72	Pyuthan	*Jhilunge*
2	NPGR-05410	Dhankuta	*Masyam*	37	LRGR-79	Dang	*Siltung*
3	NPGR-05427	Kabhre	*Masyang*	38	LRGR-82	Dang	*Siltung*
4	LRGR 3	Dadeldhura	*Gurans*	39	LRGR-84	Dang	*Siltung*
5	LRGR 8	Dadeldhura	*Gurans*	40	LRGR-88	Dang	*Siltung*
6	LRGR 14	Doti	*Gurans*	41	LRGR-89	Dang	*Siltung*
7	LRGR 25	Bajura	*Gurans*	42	LRGR-90	Dang	*Siltung*
8	LRGR 37	Achham	*Gurans*	43	LRGR-95	Nuwakot	*Khairo masyang*
9	LRGR 47	Darchula	*Gurans*	44	LRGR-97	Nuwakot	*Masyang*
10	LRGR 54	Baitadi	*Gurans*	45	LRGR-100	Palpa	*Jhilinge*
11	LRGR 73	Pyuthan	*Raiyans*	46	LRGR-108	Gulmi	*Jhilinge*
12	LRGR 83	Dang	*Jhilunge*	47	LRGR-113	Gulmi	*Jhilinge*
13	LRGR 87	Dang	*Siltung*	48	LRGR-118	Gulmi	*Jhilinge*
14	LRGR 93	Nuwakot	*Gurans*	49	LRGR-123	Palpa	*Jhilinge*
15	LRGR 94	Nuwakot	*Kalo masyang*	50	LRGR-126	Palpa	*Jhilinge*
16	LRGR 116	Gulmi	*Jhilinge*	51	LRGR-128	Palpa	*Jhilinge*
17	LRGR 120	Palpa	*Jhilinge*	52	LRGR-130	Palpa	*Siltung*
18	LRGR 124	Palpa	*Jhilinge*	53	LRGR-131	Palpa	*Siltung*
19	LRGR 135	Palpa	*Jhilinge*	54	LRGR-132	Gulmi	*Jhilinge*
20	LRGR 141	Kaski	*Masyang*	55	LRGR-133	Palpa	*Jhilinge*
21	LRGR 143	Kaski	*Masyang*	56	LRGR-134	Palpa	*Jhilinge*
22	LRGR 145	Kaski	*Masyang*	57	LRGR-136	Kaski	*Masyang*
23	LRGR 153	Kavre	*Masyang*	58	LRGR-138	Kaski	*Masyang*
24	LRGR 154	Nuwakot	*Masyang*	59	LRGR-139	Kaski	*Masyang*
25	LRGR 160	Kaski	*Masyang*	60	LRGR-142	Kaski	*Masyang*
26	LRGR-4	Dadeldhura	*Gurans*	61	LRGR-144	Kaski	*Masyang*
27	LRGR-5	Dadeldhura	*Gurans*	62	LRGR-146	Kaski	*Masyang*
28	LRGR-7	Dadeldhura	*Gurans*	63	LRGR-148	Kavre	*Masyang*
29	LRGR-9	Dadeldhura	*Gurans*	64	LRGR-151	Kavre	*Masyang*
30	LRGR-10	Dadeldhura	*Gurans*	65	LRGR-155	Nuwakot	*Masyang*
31	LRGR-18	Doti	*Gurans*	66	LRGR-156	Nuwakot	*Masyang*
32	LRGR-24	Bajura	*Gurans*	67	LRGR-157	Kaski	*Masyang*
33	LRGR-30	Bajura	*Gurans*	68	LRGR-158	Kaski	*Masyang*
34	LRGR-35	Achham	*Gurans*	69	LRGR-159	Kaski	*Masyang*
35	LRGR-55	Baitadi	*Gurans*				

Annexe 4: Agreed ricebean descriptors for characterization of the core and non-core accessions

1. Hypocotyl colour

Ten days after emergence

1	Green	4.	dark purle
2	green purple	5	mixed
3	Purple	6.	other (specify)

2. Terminal leaflet shape

1	Deltoid	5	rhombic
2	Ovate	6	obovate
3	narrowwely ovate	7	lobed
4	lanceolate	8	other (specify)

3. Terminal leaflet blade length (cm)

Recorded for the leaf at the fourth node average of ten leaflets. Use same leaflets as in 2.

4. Terminal leaflet blade width (cm)

Recorded for the leaf at the fourth node average of ten leaflets. Use same leaflets as in 2.

5. Leaf pubescence

0 Glabrous
1 Very sparsely pubescent
3 Pubescent (sparsely pubescent)
5 Moderately pubescent
6 Densely pubescent

6. Petiole colour

1	Green	4	purple
2	green with purple spots	5	dark purple
3	greenish purple	6	other (Specify)
7	green with purple lines		

7 Leaf colour

Intensity of green colour of trifoliolate leaves at 50% flowering

3 light green
5 intermediate green
7 dark green

8. Growth habit (when first pod changes colour)

1. erect (Straight and prominent main stem with few branches at narrow angles to main stem)
2. semi-erect (main stem less prominent; branches do not touch ground)
3. spreading (branches touch ground)

9 Growth pattern (when first pod changes colour)

1 determinate (apical bud of main stem develops into an inflorescence, flowering proceeds downward)
2 intermediate (apical bud continues to grow, flowering proceeds upward)

10. Twining tendency (when first pod changes colour)
0 none
3 slight
5 intermediate
7 pronounced

11. Stem colour
1. light green
2 dark green
4 light purple
4 dark purple
5 Other (specify)

12. Days to flowering
From sowing to stage when 50% of plants have begun to flower

13. Photoperiodism of flowering
(on basis of Yala/Maha ratio of days to flowering)

14. Flowering period (period from appearance of first flower to end of flowering)
1 > 35 days) asynchronous
2 31-35 days) asynchronous
3 26-30 days) asynchronous
4 21-25 days) intermediate
5 16-20 days) synchronous
6 11-15 days) synchronous
7 < 11 days) synchronous

15. Raceme position
(when first pod changes colour)
1 mostly above canopy
2 intermediate
3 no pods visiable above canopy

16. Plant height (cm)
Mean height of ten randomly selected plants

17. Days to first mature pods
From sowing to stage when 50% of plants have mature pods

18. Pod pubescence
when first pod changes colour
0 glabrous
3 puberulent (sparsely pubescent)
5 moderately pubescent
7 densely pubescent

19. Colour of upper (back) suture of immature pod
1 light green
3 Pur[ple

2	dark green	4	Other (specify)

20. Mature pod colour

1	straw	4	brown and black
2	tan	5	black
3	brown	6	other (specify)

21. Pod shattering in the field

0 absent
+ present

22. Pod length (cm)

Mean of ten mature pods taken at random

23. Number of seeds per pod

Mean number for ten pods taken at random use same pods as in 22

24. Seed shape

1. globose
2. ovoid
3. drum-shaped
4. cylindrical
5. other

25. Seed colour

1	light green	6	black
2	dark green	7	red
3	green-yellow	8	reddish brown
4	yellow	9	mottled
5	brown	10	other (specify)

26. Luster on seed surface

0 absent (dull)
+ present (shiny)

27. 1000 seed weight (g)

Weight of 100 seeds X 10

28. General uniformity of the accession

1. uniform
3 variable
5 highly variable

Annexe 5: Details of germplasm variability

ANNEXE 5.1: Germplasm evaluation conducted in India, season 2007

S.N	Name	Days to flowering	Plant height (cm)	Type of maturity	Growth habit	Maturity	Seed Colour	Seed size	Yield per plant (g)
GVT									
1	JR-1	87	112	Continuous	T	138	Light yellow	Medium	125
2	JR-2	91	118	Continuous	T	142	Brown	Small	75
3	JR-3	93	120	Continuous	T	146	Yellow	Bold	95
4	JR-4	88	110	Continuous	T	139	Grey	Medium	100
5	JR-5	92	122	Continuous	T	142	Brown	Bold	60
6	JR-6	85	106	Continuous	T	135	Yellow	Small	105
7	BR-1	84	104	Continuous	T	136	Brown	Mediun	55
8	BR-2	85	102	Continuous	T	134	Light brown	Medium	65
9	BR-3	91	100	Continuous	T	143	Yellow	Small	85
10	BR-4	85	96	Continuous	T	135	Brown	Bold	45
11	BR-5	87	106	Continuous	T	128	Variegated	Medium	60
12	BR-6	82	103	Continuous	T	125	Blackish	Bold	50
13	DR-1	92	86	Continuous	SE	145	Brown	Small	90
14	DR-2	90	82	Continuous	SE	143	Yellow	Medium	60
15	MR-1	85	110	Continuous	T	136	Yellow	Small	115
16	MR-2	87	106	Continuous	T	139	Yellow	Bold	50
Assam									
1	JCR-06-1	90	154	Continuous	SE	134	Blackish	Bold	153
2	JCR-06-2	89	146	Continuous	SE	132	Blackish brown	Bold	154
2	JCR-06-3	98	157	Continuous	SE	128	Blackish	Medium	90
3	JCR-06-4	96	157	Continuous	SE	135	Blackish	Bold	143
4	JCR-06-5	84	146	Continuous	SE	123	Reddish brown	Bold	156
5	JCR-07-1	90	148	Continuous	SE	125	Yellowish	Medium	102
6	JCR-07-2	107	168	Continuous	SE	137	Yellowish brown	Medium	98
7	JCR-07-3	87	156	Continuous	SE	127	Blackish	Medium	70
8	JCR-07-4	98	148	Continuous	SE	128	Yellowish green	Medium	87
9	JCR-07-5	98	142	Continuous	SE	138	Yellowish	Small	88
10	JCR-07-6	103	168	Continuous	SE	140	Reddish brown to Yellowish brown	Medium	115
11	JCR-07-7	91	150	Continuous	SE	132	Light Yellow	Bold	145
12	JCR-07-8	106	153	Continuous	SE	134	Blackish	Bold	143
13	JCR-07-9	100	167	Continuous	SE	137	Wine red	Bold	152
14	JCR-07-10	86	157	Continuous	SE	128	Black smoky	Bold	146
15	JCR-07-11	96	149	Continuous	SE	136	Black smoky	Medium	115
16	JCR-07-12	88	146	Continuous	SE	129	Yellowish	Medium	107
17	JCR-07-13	100	138	Continuous	SE	134	Blackish	Medium	78
18	JCR-07-14	95	136	Continuous	SE	127	Wine red	Medium	67
19	JCR-07-15	97	154	Continuous	SE	132	Greenish yellow	Medium	136
20	JCR-07-16	99	159	Continuous	SE	136	Black smoky	Bold	145
21	JCR-07-17	87	147	Continuous	SE	128	Yellowish brown	Bold	135
22	JCR-07-18	85	136	Continuous	SE	124	Reddish brown	Bold	127
23	JCR-07-19	88	146	Continuous	SE	128	Black smoky	Bold	140
24	JCR-07-20	87	147	Continuous	SE	130	Yellowish	Bold	135
Palampur									
1	RBHP – 1	76	136	Continuous	T	139	Greenish	Medium	117
2	RBHP – 2	75	108	Continuous	T	138	Red	Medium	118
3	RBHP – 3	81	97	Continuous	T	141	Brown spotted	Medium	117
4	RBHP – 4	77	107	Continuous	T	139	Grayish green	Medium	111
5	RBHP – 5	77	98	Continuous	T	142	Red	Bold	113
6	RBHP – 6	79	97	Continuous	T	147	Light brown	Bold	131
7	RBHP – 7	80	96	Continuous	T	140	Greenish yellow	Bold	115
8	RBHP – 8	82	99	Continuous	T	142	Greenish	Bold	108

S.N	Name	Days to flowering	Plant height (cm)	Type of maturity	Growth habit	Maturity	Seed Colour	Seed size	Yield per plant (g)
							yellow		
9	RBHP – 9	82	82	Continuous	SE	141	Greenish yellow	Small	111
10	RBHP – 10	88	68	Semi determinate	SE	129	Red	Medium	110
11	RBHP – 11	82	110	Continuous	T	134	Light brown	Small	118
12	RBHP – 12	81	100	Continuous	T	142	Brown	Medium	120
13	RBHP – 13	81	92	Continuous	T	131	Spotted black green	Small	115
14	RBHP – 14	81	90	Continuous	T	137	Dark brown	Medium	113
15	RBHP – 15	86	84	Continuous	T	145	Greenish yellow	Medium	112
16	RBHP – 16	85	79	Continuous	SE	146	Brown	Medium	106
17	RBHP – 17	85	75	Continuous	SE	146	Greenish yellow	Medium	107
18	RBHP – 18	85	87	Continuous	T	147	Red	Small	113
19	RBHP – 19	84	92	Continuous	T	139	Light brown	Medium	116
20	RBHP – 20	83	96	Continuous	T	135	Greenish yellow	Medium	114
21	RBHP – 22	81	113	Continuous	T	144	Greenish yellow	Medium	114
22	RBHP – 23	81	119	Continuous	T	141	Red	Medium	112
23	RBHP – 24	84	127	Continuous	T	142	Greenish yellow	Medium	112
24	RBHP – 25	86	119	Continuous	T	137	Red	Medium	113
25	RBHP – 26	87	74	Continuous	T	121	Black green	Medium	107
26	RBHP – 27	87	90	Continuous	T	124	Brown	Medium	111
27	RBHP – 28	77	106	Continuous	T	126	Blackish brown	Bold	106
28	RBHP – 29	84	89	Continuous	T	125	Greenish yellow	Medium	110
29	RBHP – 30	84	96	Continuous	T	131	Red	Medium	115
30	RBHP – 31	84	102	Continuous	T	138	Light greenish brown	Medium	110
31	RBHP – 32	85	68	Semi determinate	SE	128	Grayish	Medium	117
32	RBHP – 34	76	69	Continuous	SE	122	Spotted black	Medium	109
33	RBHP – 35	91	109	Continuous	T	134	Spotted black green	Bold	117
34	RBHP – 36	78	103	Continuous	T	124	Black	Bold	114
35	RBHP – 37	79	122	Continuous	T	145	Light brown	Bold	118
36	RBHP – 38	93	158	Continuous	T	151	Dark brown	Bold	167
37	RBHP – 40	81	70	Continuous	T	143	Spotted light green	Medium	113
38	RBHP – 41	82	103	Continuous	T	139	Greenish yellow	Medium	123
39	RBHP – 42	86	101	Continuous	T	140	Brown	Bold	129
40	RBHP – 43	78	99	Continuous	T	146	Spotted brown	Bold	112
41	RBHP – 44	80	100	Continuous	T	144	Spotted light green	Bold	112
42	RBHP-44A	79	105	Continuous	T	142	Yellowish brown	Bold	112
43	RBHP – 45	76	92	Continuous	T	138	Yellowish brown	Medium	115
44	RBHP – 46	81	130	Continuous	T	142	Spotted blackish green	Medium	119
45	RBHP – 47	78	113	Continuous	T	142	Spotted blackish green	Bold	115
46	RBHP – 48	85	125	Continuous	T	145	Brown	Bold	112
47	RBHP – 49	79	91	Continuous	T	139	Greenish yellow	Medium	115
48	RBHP – 50	85	90	Continuous	T	145	Spotted light green	Bold	111
49	RBHP – 51	85	106	Continuous	T	144	Greenish yellow	Medium	111
50	RBHP – 52	80	113	Continuous	T	145	Spotted brown	Bold	115

S.N	Name	Days to flowering	Plant height (cm)	Type of maturity	Growth habit	Maturity	Seed Colour	Seed size	Yield per plant (g)
51	RBHP – 53	78	118	Continuous	T	142	Spotted green	Bold	113
52	RBHP – 54	79	125	Continuous	T	141	Spotted brown	Small	115
53	RBHP – 55	78	82	Continuous	T	142	Spotted green	Bold	107
54	RBHP – 56	77	91	Continuous	T	140	Spotted brown	Bold	109
55	RBHP – 57	79	120	Continuous	T	140	Brown	Medium	113
56	RBHP – 58	86	130	Continuous	T	144	Brown	Medium	123
57	RBHP – 59	91	103	Continuous	T	154	Yellowish green	Medium	117
58	RBHP – 60	83	111	Continuous	T	146	Spotted brown	Bold	116
59	RBHP – 61	79	95	Continuous	T	142	Spotted brown	Bold	122
60	RBHP – 63	80	132	Continuous	T	148	Spotted brown	Bold	138
61	Check-1BRS I	82	143	Continuous	T	145	Spotted brown	Bold	121
62	Check-2BRS II	78	112	Continuous	T	142	Greenish yellow	Bold	114
63	Check-3Nainy	78	112	Continuous	T	144	Yellowish light brown	Bold	112

Annex 5.2: Gulmi, Nepal, 2007.

Core Accessions (Replication-1)

Accession No.	Accession	Height (cm)	Days to Flowering	Days to maturity	Pod length (cm)	Seeds per pod	Pods per plant	100 seed Wt	Grain yield / plant (gm)	Remarks
1	NPGR-00007	200	108.4	166	9.7	7.8	112.2	12	142.4	
2	NPGR-00008									poor growth
3	NPGR-00010	282	105.6	165.4	9.9	7.8	106	12	129.6	
4	NPGR-00012	244	108.6	164.3	9.5	7.8	140.4	7	132.8	
5	NPGR-00015	173	108.4	161.5	9.2	8.4	102.6	11	95.6	
6	NPGR-00073	216	114.8	160	8.6	8.4	175.3	7	70.8	
7	NPGR-00076	125	109.2	157.4	7.6	7.8	54.6	6	85.6	
8	NPGR-00087	165	118.8	166.7	9.1	8.8	69	6	129	
9	NPGR-00090	92	115.2	166	7.8	8.2	43	10	9	
10	NPGR-00194	147	110.4	162	8.6	8	90	9	106.2	
11	NPGR-01975	224	111.6	161	8.6	7.6	163	10	111.4	
12	NPGR-05364	204	112.4	161	8.8	7.8	62.4	10	129.4	
13	NPGR-05368	262	116	165.2	9.4	8.8	87	8	67.2	
14	NPGR-05370	14	126	165	6	6	30	6	11	
15	NPGR-05373	79	112.4	164	7.4	7.8	64.2	9	93.2	
16	NPGR-05377	103	108.4	163	7.8	7.8	91		80.8	
17	NPGR-05382	128	105	163.8	6.8	7	25.5	10	18.5	
18	NPGR-05384	93	112	107.4	9.4	9	44.8	9	94	
19	NPGR-05386	98	111.2	160	7.2	7.6	33	6	34	
20	NPGR-05391	54	111.2	160	7.8	8.2	25.6	7	6.4	
21	NPGR-05396	77	112.4	161	7.8	8.4	38.2	6	81.6	
22	NPGR-05420	149	112	160	9.4	8	108.4	10	127.4	
23	NPGR-05423	116	112	160	8.2	7.6	40.4	6	27.2	
24	NPGR-05432	60	112	153	6.6	6.6	33.6	9	33	
25	NPGR-05565									seed insufficient
26	NPGR-06591	90	112	155	8.4	7.4	45	12	94.2	
27	NPGR-06657	107	116	162	8.8	7.8	38		49.4	
28	NPGR-06756	110	118	172.5	9	9	62.5		33	
29	NPGR-07583	172	125.3	172.8	9.5	8.5	101.3	11.9	61.75	
30	NPGR-07882	66	116	155	8.3	9	35.8	6	15	
31	NPGR-08380	110	113	158.8	7.8	8	47.3	10	28.75	
32	NPGR-08382	110	113.4	161	8.8	9	58	9	70.4	
33	NPGR-09391	94	110	162	8.4	8	35.8	8	25.4	
34	NPGR-09461	114	109.2	161	7.4	8.2	52.2	8	31.8	
35	NPGR-09464	178	112	160	8	7.8	52.4	9	43.2	
36	LRGR42	147	114.6	165	9	8.6	35	24	101	
37	LRGR43	74	113.8	165	7.8	7.6	30	21	42.2	
38	LRGR44	264	113.6	169	11.1	9.3	192	15	79.25	
39	LRGR75	144	113.8	165.2	8.2	6.6	45.4	13	43.2	
40	LRGR91	132	113.4	165	7.4	8.4	54.8	8	106.8	
41	LRGR99	240	112	164	7.8	8.6	98.8		200.2	
42	LRGR101	339	130.6	180	13.3	6	64.4	10	38.8	
43	LRGR102	97	111.4	160	8	9	34.7			
44	LRGR103	183	123.7	180	13.3	8	29.7		2	
45	LRGR107	60	125.6	161.5	7	6	17	11	23.8	
46	LRGR111	450	114	160	10	6	50	10	50	
47	LRGR117	147	113.6	162	7.8	7.6	48.2	12	39	
48	LRGR129	158	112	160	7.4	7.8	51	14	82.8	
49	LRGR137	173	125.8	150.2	10.5	8.6	53.2	9	29.6	
50	LRGR152	85	113	165	8	5	32.5	10	14	
	Average	**148.9**	**114.0**	**161.8**	**9**	**7.8**	**65**	**9.9**	**66.4**	
	Maximum	**450**	**130.6**	**180**	**13.3**	**9**	**192**	**24**	**200.2**	
	Minimum	**14**	**105**	**107.4**	**6**	**5**	**17**	**6**	**2**	

Non-core accessions, Gulmi, 2007

Accession	Height	Days to flowering	Days to maturity	Pod length	Pods / plant	Seeds / pod	100 seed wt.
LRGR 3	24			5.77		5	
LRGR4	15		120	6.4		5.1	
LRGR5							
LRGR7	15.3			4.4		2.3	
LRGR8	28.6			6.7		5.6	
LRGR9	35.2		125	7.2		6.5	
LRGR10	23.5			6		7	
LRGR14	15.8			5.3		5	
LRGR18	33.5			6		7	
LRGR24	30.6			6.3		6.5	
LRGR25	17.25			5.8		3.9	
LRGR30	41.6			6.9		7.3	
LRGR35	34.3			5.8		3.8	
LRGR37	23		86	6		5	
LRGR47	21.5			7.2	7.5	7.5	
LRGR54	22			4.7		7.5	
LRGR55	15			7.1		7.5	
LRGR72	122	115.2	160	9.4	141.5	9	8
LRGR73	175	114	165	10.5	169.6	9.6	12
LRGR79	81	108.4	157	7.8	106.8	7.8	
LRGR82	54.2	118.4	161	9.7	66.8	8.8	9
LRGR83	232.5	130	180	13.5		8	10
LRGR84	62.6	112.4	162	9.9	135.4	10	9
LRGR87	61.3	109.2	155.5	7.5	76.25	8	7
LRGR88	42.4	107.2	152.4	7.3	52.6	8.2	6
LRGR89	42	110.8	154	7.2	67.8	7.8	8
LRGR90	36.4	104.4	150	7.8	22.3	9	7
LRGR93	28	104	123.333	8	12	5.7	7
LRGR94	100.5	115.2	165	8.7	68.7	8	9
LRGR95	99	106.4	160	9.5	80.8	7.8	8
LRGR97	106	112.4	161	9.1	53.6	8.6	10
LRGR100	40.5	107.6	155	8.25	36.3	8.3	7
LRGR108	30.3	114	535.25	7.5	12.5	7.3	8
LRGR113	41	115.2	157	7.4	32.8	6.6	
LRGR116	48.75	107.2	153.75	8.125	46	6.8	10
LRGR118	37.3	118.8	155	8.7	10.7	8	7
LRGR120	63.25	115.8	160	8.9	47.6	9	7
LRGR123	59.4	122.2	164	9.0	42	8	9
LRGR124	47.5	128	160	9	17	8	11
LRGR126	50	111.6	165	8.3	18	8	10
LRGR128		121.5					
LRGR130	15	124.4	160	8.5	9	8	10
LRGR131	48.7	113.2	161.667	8	21	8.3	9
LRGR132	49.6	119.6	162	8.28	33.6	7.8	10
LRGR133	76.4	113.4	155	8.56	53.4	9	11
LRGR134	69	115.2	155	8.3	50	7	9
LRGR135	67.8	110.4	155	9	33	8	10
LRGR136	65	122	162	9.96	59	8.6	9
LRGR138	41	113	160	10	16.5	8	14
LRGR139	28	110	161.7	10	19.3	8	
LRGR141	43.3	122.2	162.5	9.2	15.7	7.5	9

LRGR142	19	126.2	162.5	9.75	8	8	
LRGR143		129.2					
LRGR144	19	129.2	165	8.3	11.7	7.3	
LRGR145	26	125.2	160	10	13	8	
LRGR146		123					
LRGR148	37.5	126	160	8	7	8.5	10
LRGR151	46	122.5	160	8	13	8	8
LRGR153	30	110.8	160	6	11	6	
LRGR154	64	107.6	160	8.2	22	7.4	9
LRGR155	26	111.6		8.7	5	6	
LRGR156	65	116.2	160	7.8	9	8	
LRGR157		127					
LRGR158		128.7					
LRGR159		118					
LRGR160	110	121	165	8.5	17	7	10
Average	**50.9**	**116.6**	**164.2**	**8.0**	**41**	**7.3**	**9**
Maximum	**232.5**	**130**	**535.3**	**13.5**	**169.6**	**10**	**14**
Minimum	**15**	**104**	**86**	**4.4**	**5**	**2.3**	**6**

Annexe 6: List of Adzuki bean SSR primers pairs screened for polymorphic loci (*Han et al.*, 2005)

Code	Forward Primer (5'-3')	Reverse Primer (5'-3')	Motif	Approx. bp		Linkage
				a	b	
CEDAAG 002	GCAGCAACGCACAGTTTCATGG	GCAAAACTTTTCACCGGTAC GACC	(AAG)16	158	179	2
CEDC009	CAGCTATACATATTGTAAAC	GTTGTCAATGTACCAGTTTG	(AC)12(AT)16	143	113	2
CEDC012	TTTAAGCAGAGACAGTTGAC	CGCCATTGTTGATATTAAGC	(AC)9(AT)17	259	234	2
CEDC014	TCCATTTCCGTGTCCATCTG	TGTTATGAAGCGCCAACG	(AC)13(AT)17	176	116	11
CEDG001	ACTATGCAGAAAGACGCTCC	GGCTCTCTCTTTCTCCATTC	(AG)26	142	122	1
CEDG002	AACTGGACCTGTACCACTGG	TACAGCCTTCTTGCACCATG	(AG)16	148	154	11
CEDG003	CCACTTTCTCTTGACTTTGC	GACCAAAGTGAAGCCAAGA G	(AG)9...(AG)13	228	222	1
CEDG005	CCAGTACCCCATATTCTTCC	CTGTGTTTGGGTTGTGATGG	(AG)22	141	145	4
CEDG007						
CEDG008	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAC	(AG)26	119	101	5
CEDG011	CCCAACCAAAGCGTTTTG	CTTCTAGACTCTGAGCACTG	(AG)16 AA(AG)6	148	158	4
CEDG014	GCTTGCATCACCCATGATTC	AAGTGATACGGTCTGGTTCC	(AT)12(AG)14	137	169	5
CEDG015	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAA C	(AG)27	213	175	6
CEDG016	TTAGTTCACTCCGCTTGGTC	CACGTCATCCTCTGTTAGAC	(AG)26	176	154	8
CEDG018	AGCGTGTTTGTGGTGATAGC	ACACAGGAACGAACAAACC C	(AG)32	184	134	5
CEDG021	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC	(AG)26	165	159	10
CEDG022	AGGAATGTGAGATTTG	AATCGCTTCAAGGTCAAGCC	(AG)27	189	145	9
CEDG023	GCTCTCCATGAATGGAGTTG	TCATTCATTCACCCCTCC	(AG)16	81	79	5
CEDG024	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC	(AG)18	140	134	9
CEDG026	TCAGCAATCACTCATGTGGG	TGGGACAAACCTCATGGTTG	(AG)26	162	176	2
CEDG029	GATTGCTTTTAGCAGAGGGC	GAAGAAACCCATCTCGATCC	(AG)8	161	117	2
CEDG033						
CEDG037	GAAGAAGAACCCTACCACAG	CACCAAAAACGTTCCCTCAG	(AG)16 AC(AG)8	155	135	6
CEDG041	GCTGCATCTCTATTCTCTGG	GCCAACTAGCCTAATCAG	(AG)21	117	107	7
CEDG042	CACAGTGGTTTGGGCAACAG	TCAGAGGTTCCCATTTCCCG	(AG)15	122	162	11
CEDG043	AGGATTGTGGTTGGTGCATG	ACTATTTCCAACCTGCTGGG	(AG)14	159	191	3
CEDG044	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	(GT)10 AT(AG)18	135	138	11
CEDG050	GGCAGAATCGTACAAGTG	GTCAGATTCTCGCTTGCATG	(AG)12	131	169	2
CEDG051	AAACATACCCCTGGCAGTTCC	TTCTGACCTAAGAAAGAGCC TGG	(AG)12	235	239	1
CEDG071	GGTCCATTGAGACGGATCGAG	TCCCACCTCAGCGGAATCC	(AG)9	249	273	8
CEDG073	CCCCGAAATTCCCCTACAC	AACACCCGCCTCTTTCTCC	(AG)24	169	175	8
CEDG077	ATCCCGTGACCCTTCTTCCT	GCTCAAGCGAAAACCCAGC A	(AG)8	180	178	4
CEDG081	TGTGGGTGTTTTATGCTTTGTG	GTATTCGGTCATTCGATCTTA C	(AG)26 AA(AG)14	191	135	10

Code	Forward Primer (5'-3')	Reverse Primer (5'-3')	Motif	Approx. bp		Linkage
CEDG082	CACTCAAATAGGATTTGGTTGC	ACAATGTTGCATATCCCTTTCC	(AG)18	146	136	8
CEDG084	ATCAACTGAGGAGCATCATCGA	CAACATTTCAACCTTGGGACAG	(AG)13	168	182	3
CEDG087	CCTCTTGAAATTCTCCTTGA	CCTCTTGTGAACCTCAATAA	(AG)10	129	123	1
CEDG090	ATAAGTAGAAATTGGTTCAAATG	GGTTCGTTAAAGTAACTTTTAAT	(AG)28	157	130	1
CEDG092	TCTTTTGGTTGTAGCAGGATGAAC	TACAAGTGATATGCAACGGTTAGG	(AG)17	156	192	8
CEDG098	AAAGGAGTAGAAGGTGCATA	ACAAAATTGGTTGACTCACC	(AG)5...(AG)9	112	128	11
CEDG100	CCCATCAAGTAACTACATAACA	ATGTGGGACTGGACAAATAAAA	(AG)4 A(AG)2 A(AG)3	181	115	11
CEDG102	GCCAAGGTGAACGGTGGTG	GAGCGAGAATGGCGGAAGG	(AG)29	172	146	1
CEDG103	CACCGCTGTCCATTGAAGTATTA	TCTTAGAGTGCCCTGTGAGATTG	(AG)37	126	98	4
CEDG104	TATGGCCCGAGCAAACCTTG	CCGTTCGGTCTTCGGTTGAA	(AG)13	143	141	11
CEDG111	TGGAAGTTTCCAAGAGGGTTTTC	TCTCACCACCTTTTACCTTCTCA	(AT)7(AG)14	202	216	7
CEDG112	GCAATATTCGCATTATTCATTCA	GTGTTTCAAAGCACTATACTTAA	(AT)18(AG)20	164	168	8
CEDG114	GAACCTTGATGAAGGGGTAA	GATCACAAAGCAAAGCACAT	(AG)20(AT)8(GT)8	231	361	5
CEDG116	TTGTATCGAAACGACGACGCAGAT	AACATCAACTCCAGTCTCACCAAA	(AG)4 AA(AG)12	160	160	ND
CEDG117	GTACACTTCCACTAATCCAAAATT	TGGTACCTTCCTTATCTGAAATTA	(AG)21(AT)30	168	129	3
CEDG118	AACCCAACCAACCCTTGTGGTAAG	GCTGGAATCATAATACCGCCTTGT	(AG)21	190	130	6
CEDG121	CTTTCAAAATAATGTTGAGGCATA	CAATACATAAATAACCTTTTCTGC	(AG)18	80	86	6
CEDG124	AGCAAATTATTGGATGAAAG	TTATTTGGAATACGGATTGT	(AG)9	223	221	5
CEDG125	TGGAATATACTGTTTAATAGAG	AGATTAATTTGATCACTCATTC	(AT)14(AG)12	202	226	8
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	258	280	4
CEDG131	CCTTTTTCTTCCTACCCTCTACC	CACCACCTAGCTGTTGCTAG	(AG)12	153	171	7
CEDG134	CTCCGTGTTGAAACAATGACG	GGTCTTTCTGATCTACGAACTTG	(AG)11	217	201	10
CEDG138	CATTCTGATGAAAAGATCAAGG	CAATGTAACAGACTCACTGG	(AG)21	259	205	1
CEDG141	CCAGGCATCCATGATGACC	GAAGTTGTTGGTAATGGTTGCCTC	(AT)6(AG)13	167	179	1
CEDG146	GGTGATCGGATTTCAGAG	GGAGAAGAGAATAGAGACG	(AG)9	122	118	6
CEDG147	CTCCGTCGAAGAATTGGTTGAC	GCAAAAATGTGGCGTTTGGTTGC	(AG)10	276	330	10
CEDG150	GAAGGGAATGAAAATGAAACCC	GTTCAATCCATTCAGTCTCC	(AG)14	182	194	10
CEDG151	GTAGAACAGTTATGACACATG	TGTTAACTTCGTTGGGTACAC	(AC)6(AT)4(AGAT)3(AG)17	180	166	8
CEDG154	GTCCTTGTTTTCCTCTCCATGG	CATCAGCTGTTCAACACCCTGTG	(AG)14	212	232	4
CEDG158	GGTCAACAGGAGAGTTAG	CCACCTCCTCATTACCATTC	(AG)19	132	116	5
CEDG165	GCTCTGTCAGTTCCCACTAC	GGTCCTGAACCCAGATGAAC	(AG)10	146	142	4

Code	Forward Primer (5'-3')	Reverse Primer (5'-3')	Motif	Approx. bp		Linkage
CEDG166	GGTACAACATTCTTCTATTTG	GGCTTATGAGTTTATCTTATC	(AT)12(AG)18	228	208	9
CEDG173	GATAAGAGATGCATCACTC	CTTCTCTTCCATCACATCTG	(AG)23	124	110	9
CEDG174	GAGGGATCTCCAAAGTTCAACG G	GAAGGCTCCGAAGTTGAAG GTTG	(AG)22	215	191	7
CEDG178	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	(AG)10 G(AG)5	139	137	1
CEDG180	GGTATGGAGCAAAACAATC	GTGCGTGAAGTTGTCTTATC	(AG)11	126	122	10
CEDG183	CTCATGGTGCTACCAACCTTGA C	CCATCGCCAACGAAGTTGGT C	(AG)17	166	156	11
CEDG186	GGATGGGAGAGTAAGAAG	GCATGGCATGATGACTTG	(AG)18	180	156	3
CEDG191	CAATAAGCAATCTGTGGAGAG	CTGCAGGAAACTTGGAATTG C	(AG)21	155	149	6
CEDG195	GAGGGTCTCCACTTTTGAAACC C	GATACTAAGGCTTTCTCCACC CAC	(AG)11	141	127	6
CEDG201	CGGGTAGACAAAGAGATACAC G	CTAGCAGAAACAGGAGATCC TC	(AG)10	161	167	7
CEDG202	GTTGGAGTCTTGCACTGCG	CTATCCCCTGATCAGGAGC	(AG)12	166	182	8
CEDG203	GACTGAACCTATGCGGTCCAAC	CAACGTGTTAGCCTTCTTGCC TC	(AG)11	136	126	7
CEDG204	CCTTGGTTGGAGCAGCAGC	CACAGACACCCTCGCGATG	(AG)15	156	164	1
CEDG205	GTGGTGGTGACAGTAGCAGTAG	CAGCCACCACAAGACAACCT C	(AG)4 AT(AG)11	151	171	3
CEDG210	GAACCCACTTCTGAAGTTC	GAACAACCTCTGCAGTAG	(AG)10	188	212	2
CEDG212	CTTAAGGCAGATTACCTG	GCAACGCAAGTTATCAAG	(AG)22	250	228	5
CEDG214	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC	(AG)4 AA(AG)31	193	185	1
CEDG225	GAGGAAGTGTTGCAGCACC	GTAGACTCTGCAGAGGGATG	(AG)8 TG(AG)3(TG)2 (AG)4	143	163	2
CEDG228	GTCGTTTCCGGAAACTGTTC	GATCCGAACCTCTTTCTGC	(AG)17	197	219	9
CEDG232	GATGACCAAGGTAACGTG	GGACAGATCCAAAACGTG	(AG)16	149	173	4
CEDG238	GCAGAATTTGACTGCTAGAAAG C	CCATACATTTGTTGCACGCAT G	(AG)12	161	173	9
CEDG241	GTGACCCACTAAATTTCGTG	GAACTGGCTATTCCGGTAAC	(AG)5	260	266	1
CEDG245	GATAGAGCTTAAACCCTC	CTTTTGATGACAAATGCCC	(AC)10(AT)9(A G)14	146	196	6
CEDG247	GTAGACACTGATCATCACC	GACCATCATCGATACGATTC	(AG)16	149	169	8
CEDG248	CAGAACACAAAAGGGTTCTCG	GTGGATTCACTCGCTTCC	(AG)17	108	130	6
CEDG251	ATATCTCAAAACCCTTCCTG	CCTCAATAACAATGATACGAC	(AG)12	190	222	8
CEDG253	CACTTCCATGATGACTCACC	CACCCTTCTTTATCCTCTTCG	(AG)30	236	216	5
CEDG254	CGATGTCTCTTGCTTCAAGG	GTGAAGGACTAGCCAAGTTT G	(AT)13(AG)11	142	174	1
CEDG257	GACTACTCTCAAGACCAAAG	GATGGTTGTAGATAACACTCC	(AG)12	109	107	8
CEDG259	GATCATCGGACAGAGCTTCC	CACTCTCTGCGAACTCAATC G	(AG)11	142	138	9
CEDG263	GATTGGGAATCTGCTGTTG	GTGATCCACACACAGTAC	(AG)6 AT(AG)7	138	122	1
CEDG264	GATTCCCTTCCTAGCTATGG	CTGCTGGACATGAAGATTCA G	(AG)10 AT(AG)16	197	203	5
CEDG268	CATCTCCCTGAAACTTGTG	GCTATCAATCGAGTGCAG	(AG)16	171	145	5

Code	Forward Primer (5'-3')	Reverse Primer (5'-3')	Motif	Approx. bp		Linkage
CEDG269	CTGTTACGGCACCTGGAAAG	GCAGAGACACACCTTAACCTTG	(AG)14	190	184	8
CEDG279	GGTCTTTCTAAGCGGAGCAC	CTGCCTCTCTACACAAGTGG	(AG)5(AAAG)2(AG)3G(AG)9	184	182	11
CEDG280	CAGATTCAGTCTGCTTTTGAG	CCACTGCATTCATTCATGAG	(AG)14	175	203	10
CEDG282	CAGCAACAAGACATGGAGTG	GGTGACCACTTAGACAGAC	(AT)16(AC)5(AG)10	133	153	6
CEDG286	CGAGCAGAACACTGATCATG	CCTCTTAGAGGTCATTGCTC	(AG)23	227	205	8
CEDG287	CCTTATACTAAAGATGTTGGTGG	GTGATACGCATATAGGTTCAC	(AG)14	158	148	11
CEDG290	GACACTCTTTGTTTGTAGG	CAGTGATCACTCTGGTTG	(AG)11	139	133	7
CEDG292	GTGGTTTTGTTGACCTTGTC	GTAATGCTCCAATGGCTTC	(AG)6	160	146	4
CEDG294	CACCTTCTTAATCTCTTCACC	GGGTTTCTCTTAATTCATTGAGTC	(AT)27(AG)15	213	231	3
CEDG304	ACCACTTCATAATCCCTGAG	GTTGCATGCTATATTTTGGTTCAC	(AG)9	82	84	9
CEDG305	GCAGCTTCACATGCATAGTAC	GAACTTAACTTGGGTTGTCTGC	(AG)22	124	134	3
CEDGAG001	CTCATCAGGGACATCCTCCC	GATCGTGATCGATCCAACGGTC	(GAG)4	172	166	9

a = product size of *V. angularis*; b = product size of *V. nepalensis*; ND = not determined