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Abstract

The project Food Security through Ricebean Research in India and Nepal (FOSRIN) is a 4-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 from 1 April 2009 – 31 March 2010. Achievements and work progress are described for all relevant workpackages, and additional information provided on project management, coordination activities, and dissemination and exploitation of results. The document contains a 4-page *Publishable Executive Summary*. Annexes with detailed information on experimental protocols, germplasm characterisation, and SSR primers screened, are provided, and deliverables and other outputs are attached to the report.

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 to build sustainable medium and long term food security.

The hedonic equation was improved and finalised. Rural prices were significantly lower than prices in semi-urban areas. Consumers preferred large black seeds and high crude fibre, protein and fat, a good ratio of length to breadth. A JavaScript programme was written to allow easy comparison of varieties. Market chains were described, and farmers interviewed on post-harvest practices. A few additional samples of germplasm were collected and evaluated in India. A set of 35 polymorphic markers identified earlier were used to elucidate genetic distinctness and detect marker diversity in Nepalese and Indian germplasm. Both showed similar diversity. With one exception, the most polymorphic loci all contained (AG) repeats. D3.2, describing molecular diversity, was completed, and work continues on D3.3, to compare molecular diversity with the morphological and phenotypic diversity.

Hardseededness was irrespective of seed coat colour, and not associated with production environment or genotype. Breeding was done to purify promising populations. Mixtures of seed colours suggested that mechanisms of seed coat colour inheritance were different to other legumes. Promising entries for grain production were identified, and seed multiplied. Farmers preferred short duration, bold seeded varieties that allowed timely sowing of a following crop. We assessed the effect of sowing date at a range of altitudes. These confirmed “post-rainy-season” cropping as a possibility, but sowing in winter (November) decreased biomass and days to maturity, although some accessions showed promise for yield with good pod formation. Four ricebean accessions were tested with three blackgram. Results showed that ricebean could be a good option in the mid hills where no crop can be taken after blackgram.

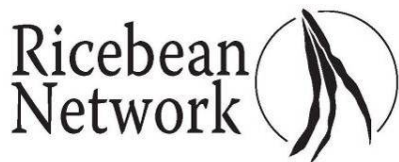
D5.1 (*Food preparation and diets*) and D5.3 (*Health & nutrition impacts*) were produced. Ricebean is most commonly eaten as *dal*, but should be soaked before cooking. Popular food items can be made from dehulled and ground ricebean and could reach a larger market and improve marketing. In the areas studied, lentils were the most important pulse overall, but there were major regional differences. Ricebean was the sixth most frequently reported pulse. The staple grains rice, wheat and maize were always the major sources of energy, protein, single amino acids, and most micronutrients, although in all surveyed sites, pulses were eaten more or less daily. Ricebean has less protein but more lysine and other essential amino acids than other pulses. It has a little less of some B vitamins (except folate) and Zn, but is high in minerals, especially Ca, Mg, K and Fe. The most widespread dietary deficiencies were vitamins: A, B₉, B₁₂, C, D and E, and Ca, Fe and K, particularly for pregnant women. For other nutrients, *mean* values were close to or above the RDI for non-pregnant women, although several B vitamins were *skewed so*

many women were below the recommendations. The most important potential role of ricebean would be with respect to vitamin B's, especially folate, and minerals, especially Ca, Fe and K.

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Executive summary



Introduction. This document describes the fourth and final year's work of the FOSRIN (Food Security through Ricebean Research in India and Nepal) 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 continued until March 2010. The work of the project is showcased on the website of The Ricebean Network (www.ricebean.org).

Background. Cereal production in S 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 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.

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:

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

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Crop Research Component, Gramin Vikas Trust, Dahod, Gujarat, India (GVT)

Ch. Sarwan Kumar Krishi Vishwa Vidyalaya, Palampur, Himachal Pradesh, India (CSKHPKV)

Assam Agricultural University, Jorhat, Assam, India (AAU)

Nepal Agricultural Research Council, Kathmandu, Nepal (NARC)

Local Initiatives for Biodiversity, Research and Development, Kaski, Nepal (LI-BIRD)

Senior staff involved

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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. A comprehensive literature review has been carried out to form part of a PhD thesis, although the review process is naturally on-going. The hedonic equation was improved by using population density to classify the locations of markets as rural, semi-urban or urban, and these variables were entered into the hedonic price analysis equation. Rural prices were significantly lower than prices in semi-urban areas. Consumers preferred ricebean with large seeds and high crude fibre contents, as well as high protein and fat content, a good ration of length to breadth, and black colour. A JavaScript programme was written to allow the easy comparison of varieties, simply using an internet browser. Market chains for ricebean in India and Nepal were described, and farmers interviewed about post-harvest practices. Results were presented in conferences in China and Germany. A PhD thesis incorporating the knowledge gained from this WP will shortly be submitted.

The second workpackage on assessing genetic diversity and indigenous knowledge is coordinated by the Nepal NGO LI-BIRD. Very little work remained in this WP. A few additional samples of ricebean germplasm were collected in various parts of India, and evaluated in on-farm and on-station trials. Work continues to write up the material to produce a journal paper. In WP3, work on molecular diversity was continued by NARC. A set of 35 polymorphic markers identified earlier were used to elucidate genetic distinctness and detect marker diversity, using 91 samples from Nepal, 25 from India and 3 checks. The range of diversity in both Indian and Nepalese samples was similar. The most polymorphic loci in both the Indian and the Nepalese germplasm were identified: with one exception these all contained (AG) repeats. Deliverable 3.2, describing the molecular diversity, was completed, and work continues to produce Deliverable 3.3, which will compare the molecular diversity with the diversity identified in WP2. This has suffered long delays due to the continuation of power outages in Nepal for up to 12 h a day.

WP4, on germplasm characterisation, is led by GVT, with inputs from the other Asian partners. A considerable amount of seed was multiplied in both countries, but in particular in Nepal.

Physiological investigation of hardseededness in Nepal showed the amount of hardseededness to be irrespective of seed coat colour, and not associated with production environment or genotype. There are some indications that ricebean has a degree of outcrossing behaviour, backed up by earlier observations. Initially, breeding was carried out to purify the population, from four accessions selected based on colour, size of seed and yield potential. At NARC, two sets, each of two accessions (dominant and recessive for seed coat colour) were studied for outcrossing behaviour, growing as pure, isolated plots or as mixtures. Even in the isolated, pure, plots a mixture of seed colours was harvested, suggesting that the mechanism of seed coat colour inheritance was possibly not as in other legumes.

In India, promising entries for grain production included JCR 51, and BRS-II, although there were significant differences between locations. Farmers preferred short duration, bold seeded varieties. In Nepal, NPGR-836 and NPGR-05364 were highest yielding. In the baby trials, farmers preferred dwarf, short duration, varieties that allowed timely sowing of a following crop.



Farmer in Ladruhi, HP, India with his ricebean



Ricebean in coordinated Indian trial on the CSKHPKV experimental farm, Palampur, Himachal Pradesh

Although ricebean is traditionally sown between May and July, sowing later is a possibility. Trials were conducted to assess the effect of sowing date at a range of altitudes. In Nepal, these confirmed “post-rainy-season” cropping as a possibility, and that vegetative growth and time to maturity were reduced without too much decrease in grain yield. Sowing in winter (November) was also investigated. In Nepal, biomass and days to maturity decreased, and some accessions showed promise for yield with good pod formation. In India, two varieties set seed, but had only a poor yield. These may be suitable for further investigation due to their obvious insensitivity to temperature and photoperiod.

Finally, a trial was conducted to compare ricebean with blackgram (*Vigna mungo*), the major summer legume in marginal areas in the mid hills of Nepal. Four ricebean accessions were tested with three blackgram genotypes. Three yielded more than 3 times the blackgram, and were taller, indicating increased total biomass production. However, ricebean was slower to mature than blackgram. Ricebean could be a good option in the mid hills where no crop can be taken after harvesting blackgram.

UB were responsible for WP5, on health and nutritional aspects of the crop. Two deliverables were submitted, D5.1 and D5.3 summarising the work. D5.1 was concerned with food preparation and diets. Ricebean is most commonly eaten as a *dal* with staple grains and vegetable curries. It is common to soak it before cooking. Since ricebean contains much phytic acid, this is essential to reduce the anti-nutritive effects that bind Ca, Fe and Zn in complexes that are not bio-available. As other nutritive parameters are affected by these processes, the trend towards pressure cooking without prior soaking/sprouting, should be discouraged. Although dehulling and processing is less common with ricebean, our trials have shown that popular food items can be made from dehulled and ground ricebean.

New products of this nature could reach a larger market and improve marketing. In the areas studied, lentils were the most important pulse overall, but there were major regional differences. Ricebean was the sixth most frequently reported pulse, which may reflect the effort already made to promote it by FOSRIN. The staple grains rice, wheat and maize were always the major sources of energy, protein, single amino acids, and most micronutrients, although in all surveyed sites, pulses were eaten more or less daily.

D5.3 contained the main findings from the dietary survey and compared it with data on nutrient contents to establish the potential role of ricebean to meet recorded deficiencies. Ricebean contains somewhat less protein but with a better content of lysine and other essential amino acids than other pulses. It has a little less of some B vitamins than the average, but not when it comes to folate. The main difference between ricebean and eight other pulses was a substantially higher content of several minerals, especially calcium,

magnesium, potassium and iron, while it contains a little less zinc than other pulses. The calcium content is the highest of all pulses. The most widespread deficiencies were vitamins: A, B₉, B₁₂, C, D and E, and calcium, iron and potassium. Recorded intakes were far below recommended values for non-pregnant women and even worse for pregnant women. For other nutrients, *mean* values were close to or above the recommended daily intake for non-pregnant women, but with considerable variations in distribution of intake, and several B vitamins were *skewed downwards so many women were below the recommendations despite mean values indicating sufficient intakes*. The most important potential role of ricebean would be with respect to vitamin B's, especially folate, and minerals, especially Ca, Fe and K.

End results

We have achieved all our expected outcomes. We have developed a hedonic demand function for legumes and completed and quantified the MLTVI, and designed a strategy to introduce ricebean into the legumes supply-chain in India and Nepal. We 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 also have a workable set of polymorphic markers for the crop which may also be of use to workers on other *Vigna* species. We know the traits that farmers regard as being important, and have identified varieties that meet those requirements. Finally, we 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

Our results will interest many audiences, such as scientists working in project-related areas, including on other grain legumes, and farmers and farmers' organisations, market traders, intermediaries and their organisations, urban and rural consumers, and policy makers. We aim for farmers in the region to adopt the new technologies and germplasm developed by the project. This is already by both informal farmer-to-farmer dissemination and by extension activities. This will have direct impact in terms of improving 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 N, 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. In the longer term the knowledge generated by FOSRIN will greatly assist the breeding of improved and well-adapted varieties of ricebean. The MLTVI will provide an exceptionally useful tool for 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 3 report, a comprehensive ricebean bibliography is available on the project website at <http://www.ricebean.org/references1.htm>. We produced eight major deliverables: distribution of ricebean in India and Nepal (D2.1); indigenous knowledge of ricebean in Nepal (D2.2); identification of polymorphic markers (D3.1); molecular characterisation of Indian and Nepalese germplasm (D3.2); diet and food preparation (D5.1); and nutrient contents of ricebean (D5.2), and D5.3 (diet and food preparation). D3.3 (Diversity estimates using agromorphological or molecular markers in relation to biophysical and socio-economic variables) is undergoing final edits. The first two deliverables from WP1 (D1.1 the Hedonic Price Function and D1.2 the Market Legumes Trait Value Index) are simple formulae and are available. The ricebean video (D7.7) has been produced and uploaded to the website and to YouTube. Many farmer leaflets and posters have been produced, and a page on ricebean added to Wikipedia. The full details of the dissemination outputs are available in the Final Report of the project.

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 maize¹, 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
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 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

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

¹ Lawn, RJ (1995)

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 1970s² 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 being described and discussed using a large number of terms: these are detailed in Deliverable 2.2⁴.

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 farmers⁵.

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 factors⁶. 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

² Chandel, KPS *et al* (1988). Ricebean - 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 agroforestry development. II: Case study on the impact of explicit representation of farmers' knowledge. *Agfor 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 agroforestry 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.

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

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 use⁷. 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 fodders⁸. 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 storage⁹, 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*), to which ricebean is very closely related^{14,15}. 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 variation¹⁶, genetic diversity in blackgram has been studied using RAPD and ISSR markers¹⁷, and in greengram using AFLPs¹⁸. Molecular markers have

⁷Sinclair, FL & Walker, DH (1998). Qualitative knowledge about complex agroecosystems. Part 1: a natural language approach to representation. *Agric Sys* **56**:341-363

⁸Thapa, B *et al* (1997) Indigenous knowledge of the feeding value of tree fodder. *Anim Feed Sci Tech.* **67**:97-114

⁹Bellon, 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

¹⁰Eyzaguirre, P & Iwanga, M (1995) Farmers contribution to maintaining genetic diversity in crops, and its role within the total genetic resources systems. P 9 – 18 in Proc Workshop on Participatory Plant Breeding, July 1995, Wageningen. IPGRI, Rome

¹¹Jarvis, DI *et al* (2000) A training guide for *in situ* conservation on farm. Version 1. IPGRI, Rome

¹²Yamaguchi H (1992). Wild and weed adzuki beans in Japan. *Econ. Bot.* **46**: 384-394

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

¹⁴Kaga A (1996). Construction and application of linkage maps for azuki bean (*Vigna angularis*). Doctoral dissertation, Kobe University, Japan.

¹⁵Tomooka N, *et al* (2003). Advances in understanding the genus *Vigna* subgenus *Ceratotropis*. Pp 25-35 in AHM Jayasuriya & DA Vaughan (eds) Conservation and use of crop wild relatives. Proc Joint Dept Agric, Sri Lanka & Nat. Inst. Agrobiol. Sci. Japan workshop. Plant Genetic Resources Centre, Dept. Agric., Sri Lanka.

¹⁶Tosti, N & Negri, V (2005) On-going on-farm microevolutionary processes in neighbouring cowpea landraces revealed by molecular markers. *Theor Appl Genet* **110**: 1275-1283

¹⁷Soufframanien, J & Gopalakrishna, T (2004) A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theor Appl Genet* **19**: 1687-1693

¹⁸Bhat, KV *et al* (2005) Amplified fragment length polymorphism (AFLP) analysis of genetic diversity in Indian mungbean [*Vigna radiata* (L.) Wilczek] cultivars. *Ind J Biotech* **4**: 56-64

been used to study genetic diversity in rice landraces in Nepal¹⁹. Twelve *Vigna* microsatellites were identified earlier²⁰, and an integrated consensus map developed for cowpea, containing over 400 markers²¹.

Linkage maps have been developed for three of the Asian *Vigna* species: mung bean (*V. radiata*), adzuki bean and black gram^{22,23} and a large number of SSR markers have been developed for adzuki bean^{24,25}. 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)²⁶. Mother trials sample more environments than replicated on-station trials²⁷, and baby trials allow the cost-effective use of many replicates²⁸, contributing major improvements over more conventional varietal testing:

- (1) Participatory trials allocate more resources to more advanced lines than many conventional breeding programmes²⁹.
- (2) Farmers can evaluate varieties for all traits and make trade-offs of, e.g., grain yield against fodder yield, maturity, and grain quality.
- (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

¹⁹ 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

²⁰ YuKang Fu *et al* (1999) Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). *Genome* **42**: 27-34

²¹ Kelly, JD *et al* (2003) Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Fld Crops Res* **82**: 135-154

²² Kaga A *et al* (2005). Molecular markers in *Vigna* improvement: understanding and using gene pools. In Lotz H, Wenzel G (eds) *Biotechnology in agriculture and forestry*, vol 55. Molecular marker systems. Springer, Berlin, Heidelberg New York, pp 171-187.

²³ Chaitieng B *et al* (2006). Development of black gram [*Vigna mungo* (L.) Hepper] linkage map and its comparison with an adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] linkage map. *Theor Appl Genet* **113**: 1261-1269.

²⁴ 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.

²⁵ Han OK *et al* (2005). A genetic linkage map for adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] *Theor Appl Genet* **111**: 1278-1287.

²⁶ Snapp, S, 1999. Mother and baby trials: a novel trial design being tried out in Malawi. In: TARGET. *Newsl. of the Soil Fert. Res. Net. for Maize-Based Cropping Systems in Malawi and Zimbabwe*. Jan. 1999. CIMMYT, Zimbabwe

²⁷ Johnson, JJ *et al* (1992). Replacement of replications with additional locations for grain sorghum cultivar evaluation. *Crop Sci* **32**:43-46

²⁸ Witcombe, JR *et al* (2005) Participatory plant breeding is better described as highly client-oriented plant breeding. I. Four indicators of client-orientation in plant breeding. *Expl Agric* **41**: 299 - 319

²⁹ Witcombe, JR *et al* (1998). The extent and rate of adoption of modern cultivars in India. In *Seeds of choice: Making the most of new varieties for small farmer* 53-58 (Eds JR Witcombe *et al*). New Delhi: Oxford IBH, and London: Intermediate Technology Publications

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 wants³⁰. 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 levels³¹, and in India only just reaches them³². 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 deficiencies³³, with around 95% of the population of S. Asia at risk of zinc deficiency³⁴. 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 heterogeneity³⁵, which requires similar heterogeneity in the innovations provided.

³⁰ Jeminez-Portugal, LA (2004) Relevant quality attributes of edible dry beans – An application of the hedonic price analysis. Osnabrück, Germany: Der Andere Verlag

³¹ Lekhak, HD (2003) "Nepal". Chapter 10 in Processing and Utilization of Legumes. Asian Productivity Organization, Tokio, Japan ISBN 92-833-7012-0

³² Govindan, A 2001: India Grain and Feed. Shopping for Pulses. GAIN Report # IN 1065. GAIN/USDA Foreign Agricultural Service

³³ Kennedy, G *et al* 2003: The scourge of 'hidden hunger': global dimensions of micronutrient deficiencies. *Food, Nutr Agric* **32**: 8-16

³⁴ Brown, KH & Wuehler, SE 2000: *Zinc and human health*. The Micronutrient Initiative, Ottawa

³⁵ Zilberman, D & Sunding, D (2001) The Agricultural innovation process: Research and technology adoption in a changing agricultural sector. Chapter 4 in BL Gardner & GC Rauuser (eds.) *Handbook of Agricultural Economics* Vol 1A, Elsevier, Amsterdam ISBN 0-4448-2588-6

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 |
|-------------------|--|-----------|---------------|
| <i>Scientific</i> | | | |
| 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 |
| <i>Management</i> | | | |
| 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 fourth reporting period, April 1, 2009 – March 31, 2010, which were as noted in Table 1.2. The timing of these milestones was altered substantially from that foreseen 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 Annual Meetings 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 fourth reporting period

| Objective | WP | Partner |
|---|----|---------|
| Improve the hedonic equation by adapting variables | 1 | CAU |
| Finalize the hedonic price analysis and make it available all project participants | 1 | CAU |
| Analysis and interpretation of surveys with farmers and intermediaries carried out in 2008 and 2009 | 1 | CAU |
| Presentation of advanced results of the hedonic price analysis to other researchers. | 1 | CAU |
| Complete collection of a representative sample of ricebean germplasm together with associated socio-economic data | 2 | LI-BIRD |
| Complete field evaluations of germplasm | 2 | LI-BIRD |
| Analyse molecular diversity | 3 | NARC |
| To complete the third year of mother and baby trials and analyse the data | 4 | GVT |
| To evaluate ricebean accessions for hardseededness | 4 | GVT |
| To carry out additional field evaluations for effect of out-of-season planting | 4 | GVT |
| To assess the extent of outcrossing, and begin population breeding | 4 | GVT |
| To multiply seed of adapted genotypes for post-project outscaling | 4 | GVT |
| To report on ricebean diets and food preparation | 5 | UB |
| To report on the potential role of ricebean in remedying food deficiencies | 5 | UB |
| To hold the 4 th Annual Meeting | 6 | CAZS-NR |
| To hold the final workshop | 6 | CAZS-NR |

Work in WP 1

This work is led by CAU Kiel, with assistance from the Asian partners. The hedonic equation was improved by using population density to classify the locations of markets as rural, semi-urban or urban, and these variables were entered into the hedonic price analysis equation. Rural prices were significantly lower than prices in semi-urban areas. Consumers preferred ricebean with large seeds and high crude fibre contents, as well as high protein and fat content, a good ration of length to breadth, and black colour. A JavaScript programme was written to allow the easy comparison of varieties, simply using an internet browser. Market chains for ricebean in India and Nepal were described, and farmers interviewed about

post-harvest practices. Results were presented in conferences in China and Germany. A PhD thesis incorporating the knowledge gained from this WP will shortly be submitted.

Work in WP2

Very little work remained in WP2, which is led by LI-BIRD. A few additional samples of ricebean germplasm were collected in various parts of India, and were evaluated in on-farm and on-station trials. Work continues to write up the material to produce a journal paper.

Work in WP3

This work is carried out by NARC, with back-up and assistance from CAZS-NR. A set of 35 polymorphic markers identified earlier were used to elucidate genetic distinctness and detect marker diversity, using 91 samples from Nepal, 25 from India and 3 checks. The range of diversity in both Indian and Nepalese samples was similar. The most polymorphic loci in both the Indian and the Nepalese germplasm were identified: with one exception these all contained (AG) repeats. Deliverable 3.2, describing the molecular diversity, was completed, and work continues to produce Deliverable 3.3, which will compare the molecular diversity with the diversity identified in WP2. This has suffered long delays due to the continuation of power outages in Nepal for up to 12 h a day.

Work in WP4

This WP is led by GVT, with inputs from the other Asian partners. Work carried out included Mother and baby trials, and combined analysis of this season and previous seasons' trials, across years and across countries. Additional work agreed for the final year was to continue evaluation of ricebean accessions for hardseededness, and additional field evaluations to assess the effects of date of planting in post rainy and winter seasons, comparisons with blackgram as an alternative legume option in Nepal, trials to evaluate population genetics and outcrossing behavior, and seed multiplication to provide material for outscaling.

In India, promising entries for grain production in the Mother and Baby trials included JCR 51, and BRS-II, although there were significant differences between locations. Good for fodder production were JR-1 in Assam and MP, and RBHP-38 and RBHP-14 in HP. Farmers preferred short duration, bold seeded varieties. In Nepal, NPGR-836 and NPGR-05364 were highest yielding. A large number of Baby trials were conducted in India. Again, farmers preferred dwarf varieties of short duration that allowed the timely sowing of a following crop of vegetables, wheat, or pulses.

Although ricebean is traditionally sown between May and July, sowing in August or even later has been shown to be a possibility. Trials were conducted to test this, and assess the effect of sowing date at a range of altitudes. In Nepal, although one site failed the trials confirmed that "post-rainy-season" cropping as a possibility, and that vegetative growth and time to maturity were reduced without too much decrease in grain yield. Sowing in winter (November) was also investigated. In Nepal, biomass and days to maturity decreased significantly, and some accessions showed promise for yield with good pod formation. In India, two varieties managed to set seed, but had only a poor grain yield. These may be suitable for further investigation confirming due to their obvious insensitivity to temperature and photoperiod. Further work showed that seed priming (soaking seed in water overnight before sowing) was inconclusive in increasing adaptation to winter sowing.

Physiological investigation of hardseededness in Nepal showed that the amount of hardseededness was irrespective of seed coat colour, and not associated with either production environment or genotype.

There are some indications that ricebean has a degree of outcrossing behaviour, backed up by earlier observations. Initially, breeding was carried out to purify the population. Four accessions were selected based on colour, size of seed and yield potential. Five superior plants from each were harvested individually, and progeny rows grown. The trials were maintained in proper isolation to avoid any outcrossing. A total of 250 plants were maintained for each accession including five progeny rows. The

best performing plants from each progeny row of the different accessions were selected (Table 2.4.13), based on plant type, pod formation, and disease and pest resistance. At NARC, two sets, each of two accessions (dominant and recessive for seed coat colour) were studied for outcrossing behaviour, growing plants as pure, isolated plots or as mixtures. Even in the isolated, pure, plots a mixture of seed colours was harvested, suggesting that the mechanism of seed coat colour inheritance was possibly not as in other legumes.

A considerable amount of seed was multiplied in both countries, but n particular in Nepal.

Finally, in WP4 a trial was conducted to compare ricebean with blackgram (*Vigna mungo*), which is the major summer legume in marginal areas in the mid hills of Nepal, but leaves the farmers few options in terms of how to grow it as it has a long growing season. Four ricebean accessions were tested along with three blackgram genotypes. Three ricebean varieties (NPGR 00008, NPGR 05364 and LRGR 91) yielded more than 3 times the blackgram, and were taller, an indication of increased total biomass production. However, ricebean was slower to mature than blackgram. The results indicate that ricebean could be a better option than blackgram in the mid hills where no crop can be taken after harvesting blackgram.

Work in WP5

Partner 5 (BU) continued analysing and reporting data from the dietary surveys. Two deliverables were submitted during the period, D5.1 and D5.3 which summarise and conclude the work of WP5. Deliverable 5.1 was concerned with food preparation and diets. The most common way of consuming ricebean is as a *dal* along with staple grains and vegetable curries, occasionally in addition to animal source foods. It is common to soak ricebean before boiling or pan frying, and in some recipes, slight sprouting is performed. Since ricebean contains a considerable amount of phytic acid, these preparations are essential to reduce the anti-nutritive effects that bind up calcium, iron and zinc in complexes that are not bio-available. Also other nutritive parameters are strongly affected by soaking, sprouting and other household level preparations. In some households may be subject to the trend of pressure cooking without prior soaking/sprouting, which should be discouraged whenever possible.

Dehulling and preparation of more processed food items are less common with ricebean, probably because the seed hull is not very easily removed. Demonstration trials have shown that popular food items can be made from dehulled and ground ricebean, so there is a need to develop methods for making new, value added products from ricebean in order to reach a larger market and improve the marketing situation.

D5.1 also contained general findings concerning the composition of diets, which had similarities in terms of being rice/pulse dominated, but also had some distinct differences. Lentils were the most important pulse overall, followed by fava bean and cowpea, but there are major regional differences. In Himachal Pradesh, chickpea is very important. Ricebean was the sixth most frequently reported pulse in the interviews, which may reflect that effort have already been made to promote ricebean in the study areas. Due to their predominance in the diet, the staple grains rice, wheat and maize were always the major source not only of energy, but also of protein, single amino acids, and most micronutrients. In all sites, pulses were eaten more or less daily: their contribution was nearly as high as that of the staple grains.

D.53 contained the main findings from the dietary survey and compared it with data on nutrient contents to establish the potential role of ricebean to meet recorded deficiencies. Ricebean contains somewhat less protein but with a better content of lysine and other essential amino acids than other pulses. It holds a little less of some B vitamin than the average, but not when it comes to folate. The main difference between ricebean and eight other pulses was a substantially higher content of several minerals, especially calcium, magnesium, potassium and iron, while it contains a little less zinc than the other pulses. Especially the calcium content is the highest of all pulses.

Regarding the deficiencies, Protein-Energy Malnutrition (PEM) was less prominent than ‘hidden hunger’ – deficiency of micronutrients. The mean values of intake indicated that the most widespread deficiencies were vitamins: A, B₉, B₁₂, C, D and E, and the minerals calcium, iron and potassium. The recorded

intakes of these nutrients were severely below the recommended values for non-pregnant women and even worse compared to the values for pregnant women. In addition, the intake of fat was low. For other nutrients, the *mean values* were basically close to or above the recommended daily intake for non-pregnant women. However, there were considerable variations in the distribution of intake of the micronutrients, and notably the distribution of intakes of several B vitamins were *skewed downwards so that many women were below the recommendations despite mean values indicating sufficient intakes*.

The *seasonal variations showed no distinctive patterns*, except for vitamin A which in some sites was abundant in the season of for instance sweet potatoes or leafy green vegetables, in other words reflecting a “real” situation. The data indicates that the most important potential role of ricebean would be with respect to vitamin B’s, especially folate, and the minerals, especially calcium, iron and potassium.

An “impact” study was made on the basis of comparing the calculated daily intake per individual in the dietary survey with what the distribution would be if 30 grams of ricebean were added, based on the assumption that this value could be indicative of a possible increase in pulse intake. This showed the *fit* between ricebean and the forecast malnutrition problems is a balance between the severity of malnutrition (mean and distribution of intakes), differences in needs between non-pregnant and pregnant individuals (and further men and other age groups), nutrient content in ricebean compared to other pulses, and the nutrient content in ricebean in particular and how it can contribute to the nutrient density of the total diet.

A final important contribution may be that the deficiencies encountered can add to the scientific understanding of what nutritional problems that can be expected in these, highly rice dominated diets. While the study confirms that micronutrient deficiencies can be more widespread than Protein-Energy-Malnutrition, some of the severe deficiencies are probably less focused in research and intervention. This relates especially to the highly inadequate intake of for instance calcium, potassium and folate.

Management activities

Routine management activities were carried out according to the workplan. The fourth annual meeting was held at Dharamshala, in Himachal Pradesh, India, in September 2009. The event was organised by Dr Kumar of CSKHPKV. The meeting updated project participants on the progress of the year 3 activities, and allowed adjustment to the plans for the final year where necessary. A number of relevant networks have been joined additional to those noted in year 3, 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. The dissemination strategy was developed during the proposal stage, and has been further refined since then. End users and intermediate users have been identified. A substantial addition was made to the ricebean page on Wikipedia, and will be updated post-project.

A large number of dissemination activities were carried out, including the large-scale dissemination of seed through Informal Research and Development (IRD). These are fully detailed in the Final Report of the project.

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. Nepal has been affected by continual power outages, at some periods for up to 12 hours a day. These have made laboratory work and data analysis very difficult.

Section 2 – Workpackage progress of the period

2.1 Workpackage 1: Supply chain, demand and marketing

2.1.1 Objectives

The overall objectives of WP 1 are to derive from an empirically estimated hedonic demand function a legume's trait value index for guiding ricebean breeding and to design a strategy for introducing an improved ricebean variety into the legumes supply-chains of India and Nepal.

Objectives / research plan for the period

1. Improve the hedonic equation by adapting variables
2. Finalize the hedonic price analysis and make it available all project participants
3. Analysis and interpretation of surveys with farmers and intermediaries carried out in 2008 and 2009
4. Presentation of advanced results of the hedonic price analysis to other researchers.

2.1.2 Summary of work in years 1-3

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 enabled 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.

In Year 2, after the literature review, a working paper on approaches to supply chain analysis for pulses in India and Nepal was 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 was 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.

In year 3, an initial version of Deliverable 1.1 – the hedonic price function – was calculated. This was presented to the International Conference on Grain Legumes held in Kanpur, India in February 2009. From this, the Legumes Trait Value Index was calculated. It was concluded that a ricebean variety that matches the needs of both consumers and farmers needed to be have large seeds, high crude fibre content, and low ash content, with high yield, drought tolerance and pest resistance. A further period of field work was carried out in India and Nepal in winter 2009, in order to sample traders and farmers who had been missed the first time round in Utrakhhand, and to sample a new area of Nepal.

2.1.3 Progress towards objectives in year 4:

(1) Improve the hedonic equation by adapting variables

To characterize the market locations where ricebean samples were collected, all markets were characterized as rural, semi-urban or urban. The population density in persons/ km² was used to classify the districts in which the markets are located in the classes of rural, semi-urban and urban. As, all markets

were scrutinized personally it was also possible to differentiate between villages and small towns, if these were located in the same district. A market in a region with a population density under 150 persons/ km² was defined as rural, semi-urban markets were defined where a population density was over 150 to 250 persons/ km² and urban markets were located where the density was over 250 persons/ km². The classification of markets in rural, semi-urban and urban was entered as dummy variable into the hedonic price analysis.

Figure 2.1.1 and 2.1.2 show box plots of ricebean prices in Nepal and India. The mean price of rural, semi-urban and urban markets is reflected by the line within the box. Both figures show that mean ricebean prices increase from rural to urban markets.

Figure 2.1.1: Price levels of rural, semi-rural and urban areas in Nepal, January to March 2008

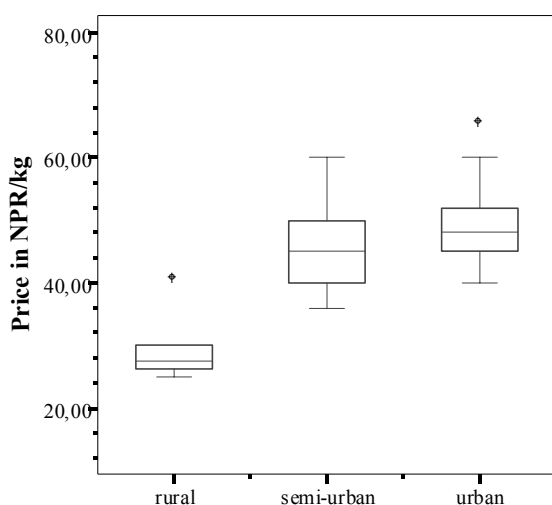
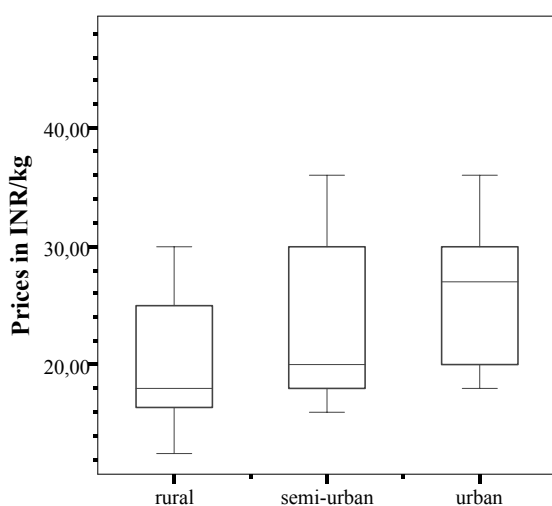


Figure 2.1.2: Price levels of rural, semi-rural and urban areas in India, January to March 2008



(2) *Finalize the hedonic price analysis and make it available all project participants*

The estimated coefficients of the hedonic model for Nepal and India are shown in Table 2.1.1. In this model for Nepal and India another dummy variable ‘country’ was introduced to differentiate between the samples of each country as these were analysed in different laboratories in each country. Thus, the data of

two laboratories were combined but the dummy was inserted to allow the model to adjust for the differences. Yet, the county dummy was not significant.

In the overall model rural prices are significantly lower than semi-urban prices but the impact for urban is not significant. Crude fibre and seed weight are significantly preferred ($\alpha=0.01$). Protein, fat, L/B-ratio and black are also preferred and show a positive coefficient with a significant impact on the price variation ($\alpha=0.05$). Protein is preferred by consumers because pulses are a cheap source for proteins in low income countries such as India and Nepal. Other sources of protein like animal products (meat) are often costlier than pulses.

An increase of the LB ratio of 1.0 indicates that a longish form is preferred by consumers because of the positive coefficient. Further, the colours olive and red are not favoured by consumers.

Table 2.1.1: Estimated coefficients and interpretation for the hedonic model for Nepal and India

Dependent Variable: \ln_price_INR

| Mean price | | | | |
|---------------------|------------------------|-------------|--------------|----------|
| Nepal & India: | | | | |
| 25.07 INR/kg | Unit | Mean values | Coefficients | T-values |
| Constant | | | 0.6578 | 0.9517 |
| Country | Dummy | | 0.0517 | 0.3011 |
| Rural ^{a)} | Dummy | | -0.2952 *** | -6.2551 |
| Urban ^{a)} | Dummy | | 0.0514 | 1.0952 |
| Moisture | % | 10.3 | 0.1762 | 1.1340 |
| Protein | % | 22.7 | 0.2763 ** | 2.1037 |
| Fat | % | 0.4 | 0.1239 ** | 1.9407 |
| CrudeFibre | % | 4.7 | 0.3500 *** | 2.3615 |
| Ash | % | 4 | -0.1304 | -0.9406 |
| Seedweight | g | 9.3 | 0.3658 *** | 5.4899 |
| Foreignmatter | % | 6.9 | -0.0118 | -0.3211 |
| L/B-Ratio | Ratio length/breadth | 1.6 | 0.4522 ** | 2.3344 |
| Swelling Capacity | Ratio volume increase | 1.3 | 0.0087 | 0.1819 |
| Black | % | 2.7 | 0.0133 ** | 2.0277 |
| Brown | % | 15.5 | -0.0053 | -0.9995 |
| Gray | % | 7 | -0.0111 * | -1.9192 |
| Olive | % | 12.6 | -0.0047 | -1.0061 |
| Red | % | 5.2 | -0.0029 | -0.5000 |
| Yellow | % | 57 | 0.0074 | 0.9211 |
| Herfindahl-Index | Colour diversity index | 0.6 | -0.0820 | -1.2112 |

R²: 0.65

adj. R²: 0.60

emp. F-value: 12.8***

N: 153

***/**/* significant at 1; 5 or 10% significance level, a) Base: semi-urban

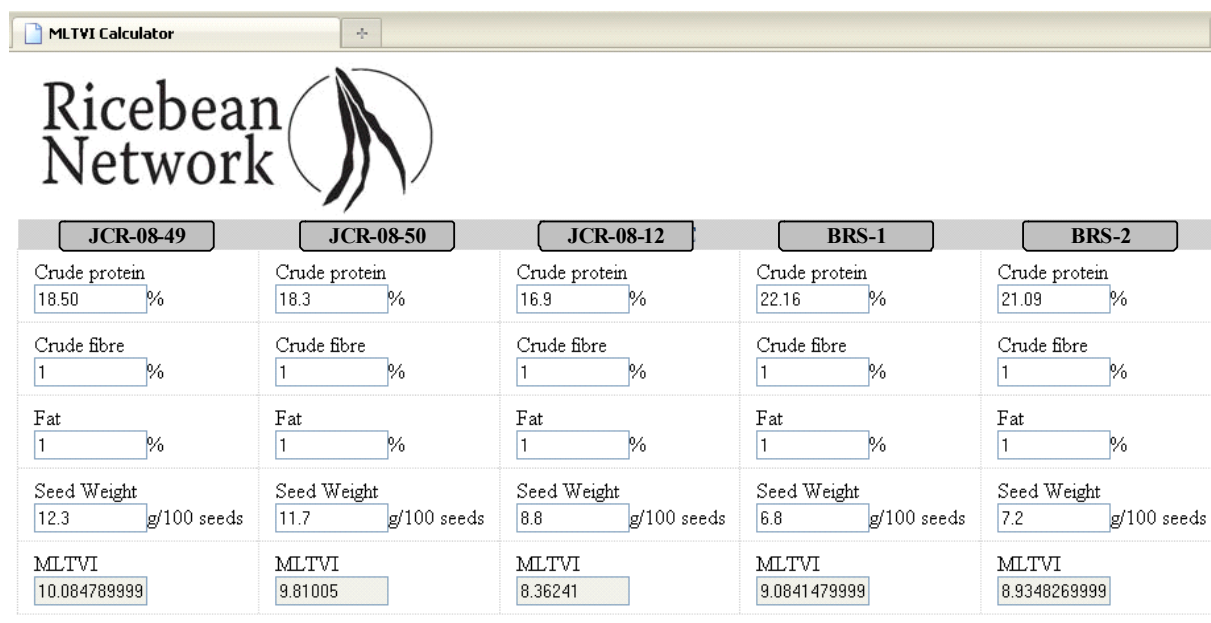
The preference of black ($\alpha=0.05$) and the dislike of gray ($\alpha=0.10$) shows that these visible characteristics may reveal other cryptic ones to consumers, which have not been included in this analysis.

To enable an easy comparison of ricebean varieties that could be chosen for breeding a program in JavaScript was written. JavaScript is an object-oriented scripting language used to enable programmatic access to computational objects within a host environment. It is implemented as part of a web browser and offers enhanced user interfaces and dynamic websites. JavaScript was designed to be easier for non-programmers to work with compared to Java.

The MLTVI can be used by breeders via an internet browser, such as Firefox or Internet Explorer. The program facilitates to enter the values of the characteristics that were found to significantly influencing the price of ricebean. These characteristics are crude protein (%), crude fibre (%), fat (%) and in seed weight (g/100 seeds). The program allows to compare five ricebean varieties at the same time. Further, limits in form of minimum and maximum values for each characteristic were determined to avoid errors while entering the values.

Figure 2.1.3 shows a screenshot of the program referred to as MLTVI calculator. In Figure 2.1.3 the characteristics of the best three ricebean varieties from Assam, JCR-08-49, JCR-08-50, and JCR-08-12, and the best two from Palampur, BRS-1 and BRS-2 were entered. Values for the characteristics crude fibre and fat are missing for those five ricebean varieties. Thus, a value of one was entered for crude fibre and fat for all five varieties to allow a comparison. As consequence, the calculated MLTVI's in Figure 2.1.3 cannot be compared to MLTVI's for which all four characteristics were determined.

Figure 2.1.3: MLTVI for four Indian ricebean varieties from Assam and Palampur



| JCR-08-49 | JCR-08-50 | JCR-08-12 | BRS-1 | BRS-2 |
|---------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Crude protein 18.50 % | Crude protein 18.3 % | Crude protein 16.9 % | Crude protein 22.16 % | Crude protein 21.09 % |
| Crude fibre 1 % | Crude fibre 1 % | Crude fibre 1 % | Crude fibre 1 % | Crude fibre 1 % |
| Fat 1 % | Fat 1 % | Fat 1 % | Fat 1 % | Fat 1 % |
| Seed Weight 12.3 g/100 seeds | Seed Weight 11.7 g/100 seeds | Seed Weight 8.8 g/100 seeds | Seed Weight 6.8 g/100 seeds | Seed Weight 7.2 g/100 seeds |
| MLTVI 10.084789999 | MLTVI 9.81005 | MLTVI 8.36241 | MLTVI 9.0841479999 | MLTVI 8.9348269999 |

The MLTVI calculator evaluates these five ricebean varieties according the presence of characteristics (crude protein, crude fibre, fat and seed weight) that are preferred by consumers. The most preferred ricebean variety of these five would be JCR-08-49, followed by JCR-08-50 and BRS-1.

(3) Analysis and interpretation of surveys with farmers and intermediaries carried out in 2008 and 2009

Data about the agents of the ricebean supply chain and their activities were obtained through interviews with legumes market experts from all stages of the supply chain and observation of transaction practices on organized legumes markets. The interviews in Nepal and India were accomplished within two years. In 2008 interviews were conducted parallel to the sample collection for the hedonic price analysis from January to March 2008. A further period of field work was carried out in India and Nepal from January to February 2009, in order to sample traders and farmers who had been missed the first time round in Uttarakhand, and to sample a new area of Nepal.

Table 2.1.1 provides an overview of the number of carried out interviews per year, country and stage of the supply chain.

Table 2.1.1: Interviews from January – March 2008 & January – February 2009

| | 2008 | 2009 | Σ | India | Nepal |
|----------------|------|------|----------|-------|-------|
| Farmer | 32 | 35 | 67 | 51 | 16 |
| Intermediaries | 63 | 53 | 116 | 67 | 49 |
| Σ | 95 | 88 | 183 | 118 | 65 |

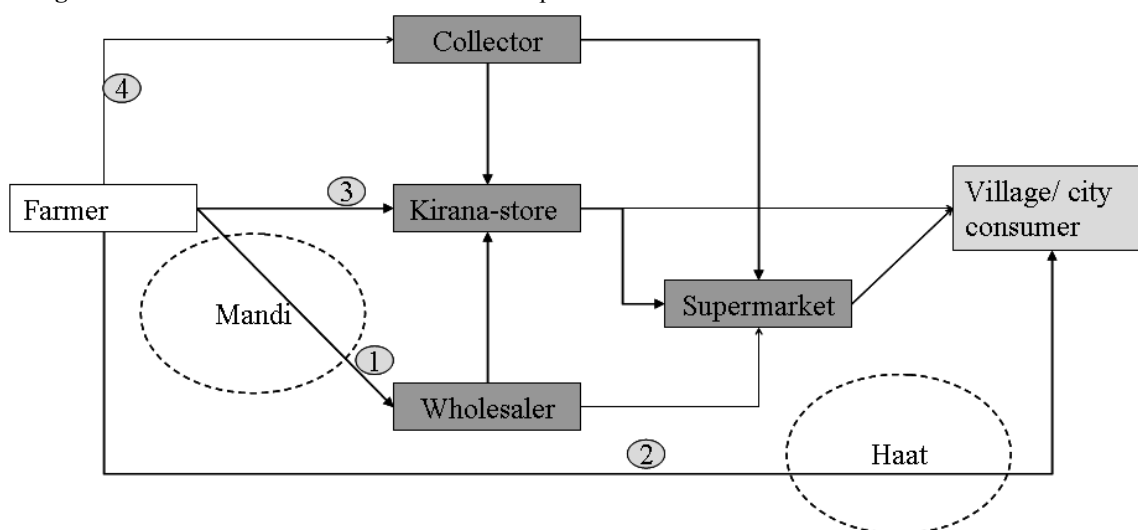
In 2008, 95 interviews had been conducted in form of semi-structured interviews. Important questions were asked to all agents of the supply chain but further answers and thoughts were also noted. These interviews provided in-depth information data about the trade of ricebean and about the data which can be obtained from farmers or retailers. In 2009, a more standardized survey was developed based on the information of 2008; 35 farmers and 53 intermediaries were interviewed (Table 2.1.1). For the following results the interviews of both years were combined wherever possible to avoid that important collected information is left idle.

To keep the order of the supply chain, we first present results of the interviews with farmers followed by the results of the interviews with intermediaries.

Farmers

In this study four different sales schemes were identified through which farmers can sell ricebean to consumers in India and Nepal. These schemes are shown in Figure 2.1.4.

Figure 2.1.4: Ricebean channels in India and Nepal



Farmers could sell ricebean at market places referred to as *haats* or *mandis*. *Mandis* are regulated markets that are mostly primary wholesale markets and controlled by APMC Act (Agricultural Produce Marketing Committee) in India. There are about 7,500 *mandis* in India which are usually situated near to important hubs of production, towns and district headquarters. Farmers could sell directly or through *mandis* to wholesalers. This scheme is labelled with (1) in Figure 2.1.4.

The unregulated markets referred to as *haats* are the second way (2) for farmers to sell ricebean. There are about 20,250 to 47,000 *haats* in India and farmers could sell via *haats* directly to consumers. The third way (3) to sell ricebean is the retail stage that includes *kirana*-stores and supermarkets. *Kirana*-stores are permanent retail outlets like “mom & pop” stores. *Kiranas* can be specialised in selling certain goods

such as pulses, however, the great majority trade in almost everything from food to hygiene products to toys. *Kirana*-stores are purchasing from farmers, wholesaler and collectors. Supermarkets are emerging rapidly in developing countries in the last decade. In this study supermarkets were identified as a source for ricebean in Nepal but not in India. The fourth alternative (4) for farmers to sell ricebean is commission agents (collectors). Figure 2.1.4 shows that there are further possible links between the intermediaries which are not described in detail.

Table 2.1.2 shows some characteristics of farmers that grown ricebean. The yield varies over a wide range what could be explained through farmer's statements about the bad weather conditions in some regions or the variance results from the cultivation type.

Table 2.1.2: Ricebean yield per farmer and seeds sown out in India and Nepal

| Ricebean | N | Minimum | Maximum | Mean |
|-------------------|----------|----------------|----------------|-------------|
| Yield in kg | 67 | 0.5 | 350.0 | 44.1 |
| Seeds in kg | 66 | 0.1 | 25.0 | 2.3 |
| Ratio yield/seeds | 66 | 0.1 | 200.0 | 29.6 |

Farmers were also asked about the post-harvest practices of ricebean (Table 2.1.3). All of the interviewed farmers stated that they are drying and cleaning ricebean and about one third also sorts them. Another third of the interviewed farmers is protecting ricebean against pest while storing. Many farmers use home remedies like ash while storing. About 70% of farmers are able to store ricebean.

Table 2.1.3: Post-harvest practices of farmers

| | Drying | Cleaning | Sorting | Pest protection | Storing |
|---------|---------------|-----------------|----------------|------------------------|----------------|
| Valid % | 100 | 100 | 28.6 | 29.4 | 70.6 |

N:35

Table 2.1.4 shows the respective share of farmers that do sell ricebean, indicating that more than half (57%) of the farmers are not selling ricebean. However, there are huge differences between Nepal and India with 88% and 29% respectively. If farmers are not selling ricebean they keep it for their own consumption. Table 2.1.5 shows that none of the famers sold the total harvest of ricebean. They kept at least 5% for their own. The mean value of about 72% for home consumption indicates that the ricebean is not primarily grown to earn income.

The distance to the next selling point which does not have to be a market can be very different.

Table 2.1.4: Share of farmers that sell ricebean

| Sell ricebean | India | Nepal | Total |
|----------------------|--------------|--------------|--------------|
| No | 36 | 2 | 38 |
| Yes | 15 | 14 | 29 |
| Total | 51 | 16 | 67 |

Some farmers can sell to their neighbours or have a local market place in their village others have to overcome 80 km to reach the nearest market place. That was a farmer from Ramechhap in Nepal who needed two days to arrive at the market.

Table 2.1.5: Characteristics of ricebean selling farmers

| | N | Minimum | Maximum | Mean |
|---------------------------------|----|---------|---------|------|
| Homeconsumption % | 68 | 5.0 | 100.0 | 71.6 |
| Quantity of ricebean sold in kg | 68 | 0.0 | 290.0 | 24.7 |
| Distance to market in km | 27 | 0.0 | 80.0 | 15.2 |

Intermediaries

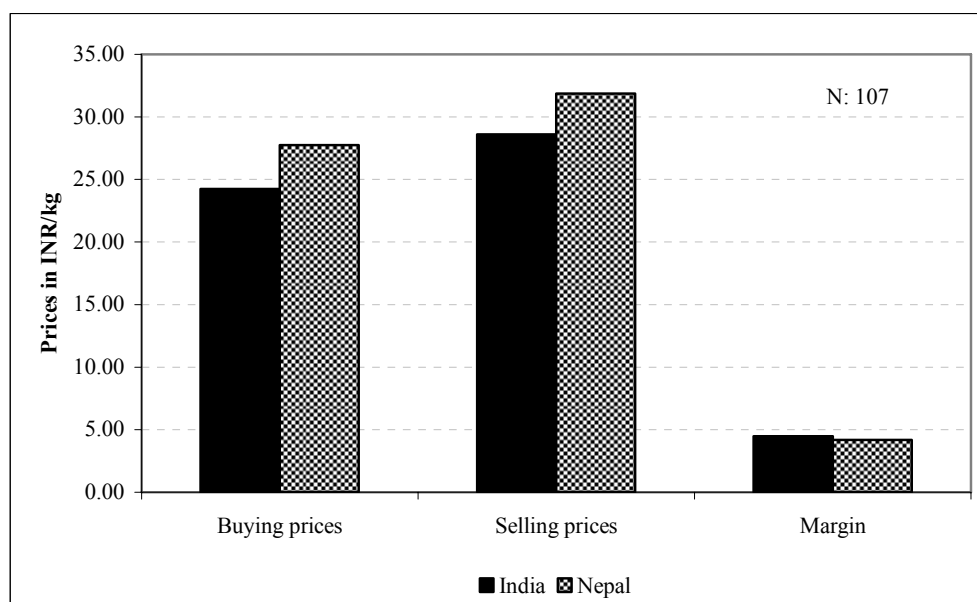
In this study the following intermediaries were identified: collectors, wholesalers and supermarkets and *kiranas* at the retail stage. As, the ricebean is not processed there were no mills or other processing firms involved.

Table 2.1.6: Number of interviewed intermediaries per stage, 2008 and 2009

| Country | Supermarkets | Kiranas | Wholesalers | Collectors |
|---------|--------------|---------|-------------|------------|
| Nepal | 3 | 32 | 13 | 1 |
| India | 0 | 56 | 4 | 6 |
| | 3 | 88 | 17 | 7 |

Table 2.1.6 presents the number of all interviewed intermediaries in Nepal and India. Supermarkets that sold ricebean were only found in Nepal. All remaining stages were present in both countries.

Figure 2.1.5 shows the price differences of mean buying and selling prices of ricebean and the received margin. In comparison to India, the Nepalese intermediaries were paying higher prices per kg for ricebean to their suppliers and sell ricebean for higher prices to their customers. Thus, the mean margin of Indian and Nepalese intermediaries is almost equal; there is only a difference of 0.31 INR.

Figure 2.1.5: Price differences of ricebean between India and Nepal

As every stage has its marketing costs, there are several mark-ups in the supply chain from farmers to consumers. Farmers at the first stage were selling ricebean for about 20.00 INR/kg in Nepal and India. The stages in Figure 2.1.6 are arranged according their mean buying and selling prices of ricebean starting with the lowest prices the following ranking emerges: collectors, wholesalers, supermarkets and *kiranas*.

Collectors were buying and selling ricebean for the lowest prices but they had the second highest margin of 4.25 INR/kg. Wholesalers were offering and buying ricebean for the second lowest prices. *Kiranas* had the highest mean buying and selling prices and the highest margin. Supermarkets offered cheaper ricebean than *kiranas*. Supermarkets also had the lowest margin per kg (2.50 INR) for ricebean.

Figure 2.1.6: Price levels of ricebean stages of India and Nepal

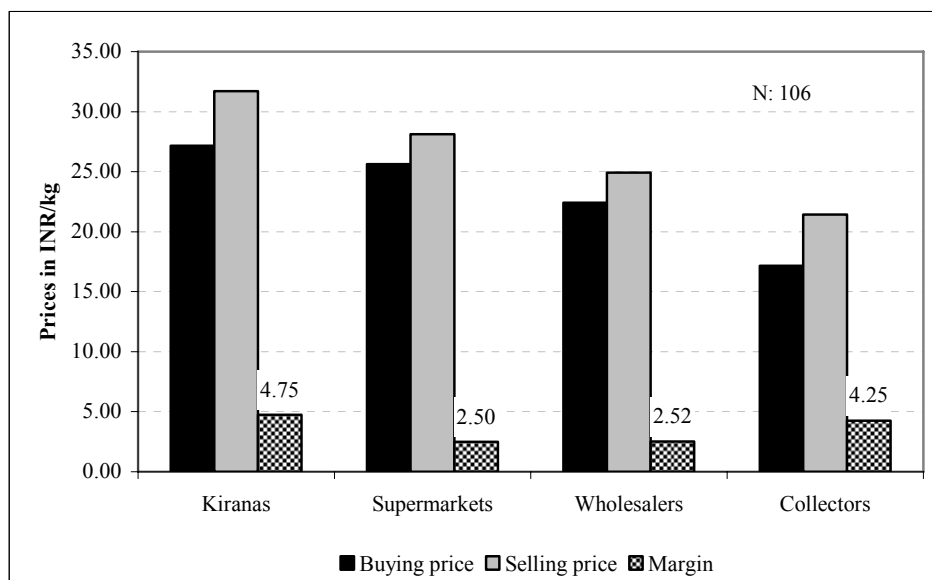
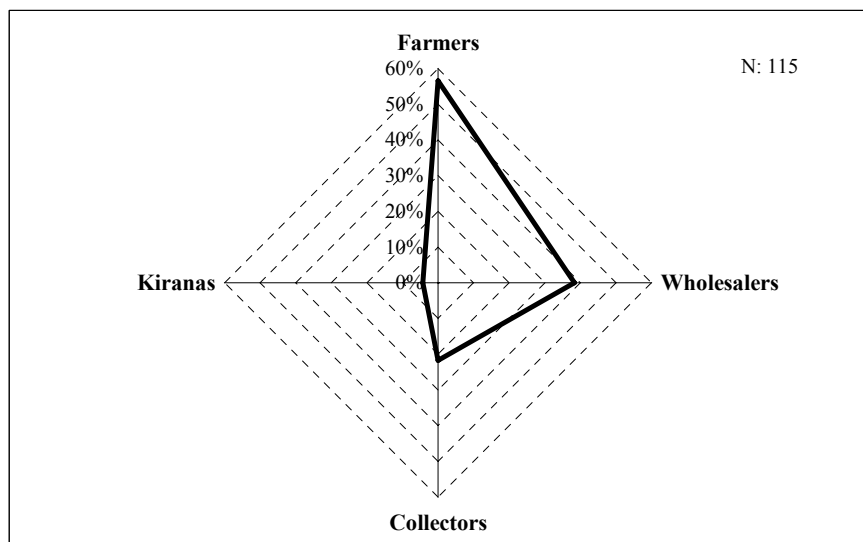


Figure 2.1.7 shows that about 60% of all intermediaries are buying directly from farmers. The second important source for ricebean are wholesaler and only about 5% are buying ricebean from *kiranas* which is reasonable as consumers are not included in this study.

Figure 2.1.7: Sources of ricebean of all stages in Nepal and India



To depict the product flow it is necessary to document the sales and purchases of every stage. Table 2.1.7 shows from who supermarkets, *kiranas*, wholesalers and collectors are buying ricebean. The first column contains the sources (seller) of ricebean and the remaining four columns show the per cent which the

particular intermediary is buying from each source respectively. The intermediaries could give multiple answers, as it was common that they have several sources for ricebean.

Table 2.1.7: Sources of ricebean of each stage in Nepal and India

| Source | Supermarkets (N:3) | Kiranas (N:87) | Wholesalers (N:17) | Collectors (N:7) |
|-------------|--------------------|----------------|--------------------|------------------|
| Farmers | 0% | 60% | 41% | 71% |
| Wholesalers | 67% | 39% | 41% | 14% |
| Collectors | 0% | 16% | 59% | 14% |
| Kiranas | 33% | 3% | 0% | 0% |

upermarkets were buying ricebean mainly from wholesalers (67%) but also from *kiranas* (33%). It was shown in Figure 2.1.6 that *kiranas* were the most costly source of ricebean. Maybe supermarkets revert to *kiranas* if the wholesalers were not able to provide ricebean. There are several important festivals in Nepal that are strongly connected with the consumption of ricebean. Additionally, ricebean is consumed at wedding parties in Nepal and there is a wedding season in the beginning of every year (February – March). Thus supermarkets have to offer ricebean at that time.

Kiranas were buying most of their ricebean directly from farmers (60%). As *kiranas* are offering a great range of products farmers could spend their money from ricebean sales for everyday commodities that are not available in villages or from wholesalers.

Wholesaler had a wide range of sources for ricebean. They are buying directly from famers or from collectors and even from other wholesalers but the main source are collectors (59%). Maybe wholesalers have the claim to offer a range of pulses and thus they use several sources of ricebean.

Collectors major ricebean source are farmers. The occupation of a collector can be described as follows: first the collector receives an order with the needed pulses, than he and his client agree on quantities and prices, afterwards he goes to villages to collect the required pulses. Some collectors receive the money in advance. As, collectors have already fixed the selling price they will try to increase the margin by bargaining about the buying price.

After describing who are the sellers of ricebean Table 2.1.8 describes who the purchasers of ricebean are. For that purpose intermediaries were asked to whom they are selling ricebean. The customers of supermarkets (100%) and of *kiranas* (83%) are mainly households referred to as end consumers. Some *kiranas* were also delivering ricebean to wholesalers, collectors and other *kirana* stores. Wholesalers were mainly serving *kiranas* (88%) but they are also directly selling to consumers. Maybe consumers prefer wholesalers as they are offering lower prices (see Figure 2.1.6). Collector's clients are *kiranas*, households and wholesalers.

Table 2.1.8: Purchasers of ricebean for each stage in Nepal and India

| Purchaser | Supermarkets (N:3) | Kiranas (N:87) | Wholesalers (N:17) | Collectors (N:7) |
|-------------|--------------------|----------------|--------------------|------------------|
| Households | 100% | 83% | 59% | 43% |
| Farmers | 0% | 0% | 0% | 0% |
| Wholesalers | 0% | 3% | 59% | 43% |
| Collectors | 0% | 1% | 0% | 0% |
| Kiranas | 0% | 9% | 88% | 43% |

able 2.1.8 also shows that none of the intermediaries was selling to a farmer which is reasonable as farmers are producing ricebean.

In conclusion, a great share of ricebean is traded in a short channel from farmers over *kiranas* to consumers. Another great share goes from farmers to collectors.

A detailed analysis of the surveys is presented in the PhD thesis of D. Buergelt that will be published as part of the FOSRIN project.

(4) Presentation of advanced results of the hedonic price analysis to other researchers.

The results were presented to a scientific audience at the 27th Conference of the International Association of Agricultural Economists (IAAE), Beijing, China, 16. August - 22. August 2009 and at the Tropentag, Hamburg, Germany, 06. October- 08. October 2009 (See Annexe 1).

2.1.4 Deviations from the workprogramme

None reported

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 NARC, GVT, CSKHPKV and AAU, 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

The majority of the work in this WP was completed by the end of year 3. In year 4, some additional germplasm collection and evaluation was conducted in India.

2.2.2 Summary of work in years 1-3

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 agromorphological 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 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.

In 2008, germplasm collection continued as an adjunct to other activities. Evaluation trials were conducted at two field sites in Nepal by LI-BIRD, in Darbar Devasthan 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 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.

In year 3, further samples of ricebean germplasm were collected in various parts of India. Representative samples of ricebean along with associated local knowledge were collected from different parts of Nepal. 54 more lines were collected from farmers' households in different areas of Northern India during winter 2008.

A large number of observation nurseries were sown for the evaluation of genotypes. Two sets of observation nurseries were conducted by LI-BIRD with 21 accessions in each set of trials. LRGR 44 (2333 kg/ha) exhibited highest yield followed by LRGR 135 (2190 kg/ha) and LRGR 129 (1956 kg/ha) in the first set and LRGR 139 (1953 kg/ha), NPGR 05422 (1722 kg/ha), NPGR 05372 (1696 kg/ha), LRGR 87 (1672 kg/ha), and NPGR 05383 (1622 kg/ha) were best among the 21 accessions in the second set of observation nursery. In trials at NARC ranging from lowland through mid- to high-hill environments (Khumaltar, Dolakha and Rampur), a number of high yielding lines were identified for promotion to yield trials in the next season. In a nursery for the evaluation of offtypes it became apparent that, contrary to accepted belief, there is substantial out-crossing in ricebean. This could have significant implications, but also present opportunities, for breeding new varieties. In India, a large number of genotypes were evaluated by the three partners, each at several sites, and a number of promising lines identified. A total of 249 lines were evaluated in *Kharif (summer)* 2008 (rainy season) for quantitative characters under farmer's field situation for isolation of promising lines suitable for cultivation in the region.

GPS coordinates were obtained to go with the distribution data collected earlier, and this work is now being written up as an academic paper. Deliverable 2.2, on Indigenous Technical Knowledge, was completed.

2.2.3. Activities of workpackage 2

Germplasm collection

During the report period, there were no formal exploration trips for collecting germplasm but during the visit to Orissa, six collections were made based on seed size, which was quite distinct from others. These were evaluated along with the old germplasm pool available with GVT. A total of 24 accessions were collected from Himachal Pradesh (10) and Uttarakhand (14) after the exploratory visits made during 2008 in ricebean growing areas of these States.

Germplasm Evaluation

A number of lines were evaluated by all the Indian partners in this reporting period. A total of 50 accessions including JR-1 were evaluated for morphological traits in farmers fields in Bhagor village, in Jhabua district of MP. The experiment was conducted during *Kharif-* 2009 in an augmented design. Evaluation was under farmer managed conditions. Each line was grown in 3 rows of 2.40 m length with

row to row and plant to plant spacing of 45 cm and 15 cm, respectively. Harvesting was at variable dates depending upon the maturity of the lines, which varies from October to December. A field experiment was also conducted at the experimental farm of CSK Himachal Pradesh Agricultural University, Palampur. One hundred and five lines along with three checks (BRS-I, BRS-II and Nainy) were tested in an augmented design. The crop was sown on 30 June 2009. The treatments were randomized within blocks. Plot layout and spacing was as in the GVT trials, but the recommended package of practices was followed. The recommended fertilizer doses (N: P: K at 20: 40: 20 kg/ha) were used at the time of sowing. The herbicide Targa super (Quizalofop-Ethyl 5% EC) was used as a post emergence spray for the control of broad leaf weeds, and plants were staked. At the flowering stage insecticides (Chlorophyrophos and Cyohalothrin), were sprayed to control blister beetle. The crop was harvested when mature, which again varied from October to November. An additional 80 lines were evaluated by AAU in Assam.

Observations in both trials were recorded on five randomly selected plants for following traits except for days to 50% flowering for which data were recorded on plot basis. Analysis of variance was carried out based on the mean of five plants.

1. Days to 50% flowering
2. Flowering period
3. Days to first mature pods
4. Terminal leaf blade length
5. Terminal leaflet blade width
6. Plant height (cm)
7. Pods per plant
8. Pod length (cm)
9. 100-seed weight (g)
10. Seed yield per plant (g)
11. Pod duration

The range of traits for the germplasm evaluated at CSKHPKV is shown in Table 2.2.1. Of the 51 lines evaluated by GVT, JR-6, JR-12, JR-44 and JR-45 gave the maximum grain yield per plant. The mean performance of lines for different characters is given in Annex 1. At CSKHPKV, GVT and AAU promising lines were identified and selected for different characters (Annex 2). The germplasm was grouped based on traits such as height, flowering period, pod duration, and grain yield (Annex 3).

Table 2.2.1. Range of phenological traits, yield traits and yield of germplasm evaluated at CSKHPKV during 2009

| Trait | Range |
|-------------------------------|------------------|
| Days to 50% flowering | 72 - 92 days |
| Flowering period | 25 - 56 days |
| Days to first mature pod | 95 - 118 days |
| Terminal leaflet blade length | 6.7 - 11 cm |
| Terminal leaflet blade width | 4.3 - 8.7 cm |
| Plant height | 45 - 147 cm |
| Pods per plant | 15 - 126 |
| Pod length | 6.5 - 10.2 cm |
| Pod duration | 13 - 49 |
| 100-Seed weight | 3.09 - 8.21g |
| Seed yield | 0.32 - 2.15 t/ha |

2.2.4. Deviations from the workplan

Most activities were accomplished according to the schedule, and there were no serious problems.

2.3 Workpackage 3: Molecular markers

2.3.1 Objectives

WP3 addresses the characterization of ricebean diversity using molecular markers, and relates the genetic diversity to agro-morphological and biophysical diversity. It is led by NARC, with assistance from CAZS-NR. A series of field- (as part of WP 2 and WP4) and laboratory-based studies on genetic diversity of Nepalese ricebean germplasm was carried out on-farms and stations in different agro-environments of the country: the field studies are reported under WP2 and WP4 as appropriate. The SSR molecular marker diversity study was carried out on a stratified test sample of landraces with a consideration of geographical and phenotypic diversity to determine the extent and amount of diversity in ricebean germplasm from Nepal and India. The relationships and diversity between the genotypes is determined and compared and related to agro-morphological and biophysical diversity for protein and fat content values determined useful in describing the genotypes at the local level. Table 2.3.1 summarises the activities undertaken to achieve the respective deliverables in connection to objectives of WP3 and related activities to other WPs .

A set of 35 adzuki bean SSR markers were earlier found to amplify ricebean DNA, and exhibited polymorphism in a stratified sample of ricebean. For the molecular diversity, a set of 91 accessions based on analysis of agro-morphological diversity were identified and the 13 most polymorphic adzuki bean SSR primers distributed across the genome were tested along with adzuki bean as check sample.

Table 2.3.1: Summary of activities, objectives and deliverables for WP3 (2009)

| Objectives | Activities performed | Outcomes |
|--|---|--|
| Analysis of molecular marker diversity | 91 accessions from 35 districts based on agro-morphological diversity selected for molecular marker diversity assay 25 accessions from India (Assam, Himanchal Pradesh and Gujarat) were also used for diversity assay Bulk DNA (15 individuals) for each of these stratified sample isolated 35 polymorphic loci useful for detecting genetic diversity were tested | Germplasm were found diverse and generated the molecular data. The molecular diversity compared with agro-morphological diversity. The observed marker diversity is analysed to relate with biophysical and socio-economic traits. |

Progress/Achievement

Farmers classify their ricebean 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. A preliminary evaluation during the first year of the project showed the existence of diversity in ricebean germplasm that could be useful for breeding improved varieties with desired traits. One of the activities foreseen for FOSRIN was an agro-morphological and DNA-based molecular diversity study to analyse the genetic diversity of local rice bean germplasm. In Work Package 3 (WP3), the agro-morphological diversity and genetic variability study of local rice bean germplasm for physiological traits (kernel hardseededness) and inheritance of seed colour trait under population genetics, and SSR diversity analysis were carried out in field and laboratory conditions in 2010. The objective was to determine the agro-morphological diversity of the germplasm, to analyze the molecular marker data using SSRs, and to identify the polymorphic loci (markers). These informative and polymorphic primers for these loci were used to investigate the molecular diversity in local ricebean germplasm of India and Nepal.

Materials and methods

Analysis of molecular marker diversity

35 polymorphic SSR markers identified in Deliverable 3.1 (D3.1) were used to elucidate genetic distinctness and detect the marker diversity in ricebean germplasm, using 91 stratified samples of ricebean from Nepal, 25 from India and three controls: adzuki bean and ricebean bold (Annex 4). The set was expected to be highly diverse to capture the total diversity in Nepal and India.

Bulk DNAs of 15 seeds of each accession were isolated using a Phytopure Genomic DNA extraction kit (Tepnel Sciences PLC, Manchester, UK). The DNA isolates were checked for 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 min with ethidium bromide staining. The thermal cycling of PCRs (polymerase chain reaction) carried in MJ Research PTC– 100TM Programmable Thermal Controller (MJ Research, INC, Waltham, MA, USA), amplified PCR products separation and diversity analysis of generated marker data for test samples were carried following the protocol described in Annex 4^{36, 37}.

Results

Analysis of molecular marker diversity in ricebean accessions

Thirty five SSR primers identified as polymorphic markers in D3.1 were tested on 112 stratified samples of ricebean of Nepal (91 accessions) and India (25 accessions) to measure molecular marker diversity and relationships in ricebean germplasm (Annex 4). Adzuki bean (red bean), bold ricebean and small seeded adzuki bean locally known as “*Gurans*” were included as checks to observe the allele variations on stratified ricebean germplasm of the study. The diversity indices calculated based on the allelic data showed a range of genetic variation among the samples and they are summarized in Tables 2.3.2 and 2.3.3. A total of 69 alleles (bands) revealed for 35 primers across 112 plus 3 check samples (data not shown). Based on the diversity values, the germplasm of Nepal and India are both equally diverse which ranged from 0.03 to 0.63 in Nepalese ricebean and 0.08 to 0.70 in Indian ricebean with averages of 0.25 and 0.27 respectively (Table 2.3.2). CEDG044 was found most polymorphic with highest PIC values (0.63) for Nepalese germplasm while it was CEDG073 (0.70) with 4 alleles in 25 ricebean genotypes from India.

³⁶ Wang XW, Kaga A, Tomooka N and Vaughan DA (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.

³⁷ Somta P, Kaga A, Tomooka N, Kashiwara K, Isemura T, Chaitieng B, Srinives P and Vaughan DA (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.

Table 2.3.2: Summary of SSR Diversity values calculated for ricebean accessions from Nepal and India

| Diversity parameters | Ricebean germplasm | |
|----------------------------------|--------------------|-------|
| | Nepal | India |
| Total sample analysed | 91 | 25 |
| Total SSR markers analysed | 35 | 35 |
| Total polymorphic loci | 30 | 22 |
| % of polymorphic loci (PPL) | 86 | 61 |
| Total number of alleles | 80 | 46 |
| Alleles per locus | 2.2 | 1.9 |
| Total polymorphic alleles | 75 | 46 |
| Alleles per polymorphic locus | 2.5 | 2.1 |
| % of polymorphic alleles (PPA) | 94 | 78 |
| H ⁺ (diversity index) | 0.25 | 0.27 |
| Shannon Weaver Index (SW) | 0.47 | 0.48 |

Table 2.3.3 lists the SSR loci found to be most polymorphic in ricebean germplasm from Nepal and India. All these contained the AG repeats except CEDAAG002 that had AAG repeats and CEDG044 that had GT(AT)AG repeats. These polymorphic primers belonged to linkage groups 1, 2, 3, 6, 8, 10 and 11 of the ricebean genome. These primers explained the variation at DNA level among the tested ricebean genotypes, and also between adzuki bean and rice bean. The molecular diversity in ricebean germplasm from Nepal and India was determined and their relationship plotted based on a principal component analysis (PCA) of the molecular data, which explained 46.3 % of the total variation (Figure 2.3.1).

Table 2.3.3: Polymorphic SSR primers with high diversity indices above average value

| Primers | Nepalese germplasm | | | Indian germplasm | | |
|-----------|--------------------|------------|---------------|------------------|------------|---------------|
| | Alleles (bands) | PIC values | Shannon index | Alleles (bands) | PIC values | Shannon index |
| CEDAAG002 | 2 | 0.31 | 0.55 | 2 | 0.56 | 0.96 |
| CEDG015 | 3 | 0.45 | 0.84 | | | |
| CEDG033 | 4 | 0.50 | 1.06 | 2 | 0.42 | 0.71 |
| CEDG044 | 3 | 0.63 | 1.15 | 2 | 0.44 | 0.65 |
| CEDG071 | | | | 2 | 0.44 | 0.63 |
| CEDG087 | | | | 2 | 0.40 | 0.59 |
| CEDG073 | 2 | 0.51 | 0.84 | 4 | 0.70 | 1.45 |
| CEDG090 | 3 | 0.56 | 0.94 | 2 | 0.27 | 0.44 |
| CEDG141 | | | | 3 | 0.70 | 1.36 |
| CEDG204 | 3 | 0.28 | 0.49 | | | |
| CEDG214 | | | | 2 | 0.50 | 0.60 |
| CEDG305 | 3 | 0.30 | 0.70 | | | |
| CEDG178 | 2 | 0.46 | 0.65 | | | |

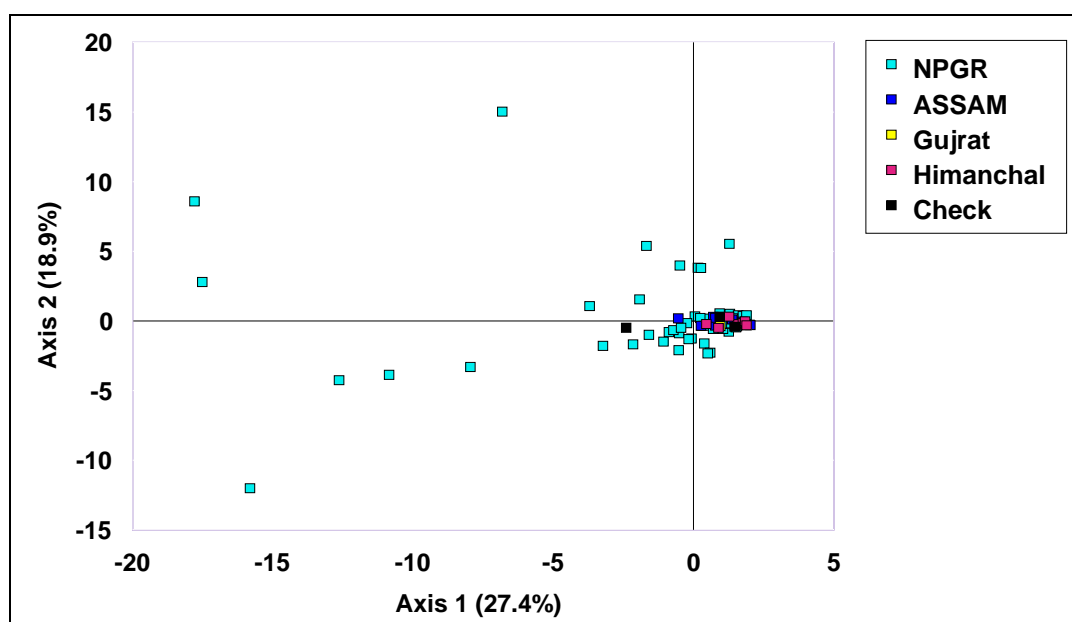


Figure 2.3.1: Scatter plot of ricebean accessions from Nepal and India showing diversity based to PCA on 79 alleles generated by 35 primers

The molecular data was compared with agronomic traits and also with biophysical (fat and protein) traits in order to produce deliverables D3.2 and D3.3.

References

- ADB (2004). Annual report of Agriculture Botany Division, NARC, Khumaltar, Lalitpur, Nepal
Anonymous (1995). Descriptors for grain legumes and oil seed crops. The Plant Genetic Resources Centre, Department of Agriculture, Gannoruwa, Peradeniya, Sri Lanka.

Deviations from the workplan

Serious and frequent power outages in Nepal have again disrupted the work this year, and led to unavoidable delays in the production of Deliverables 3.2 and 3.3

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, with NARC (Part 7) and Local Initiatives for 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 2.4.1.

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

| Objectives | Worked performed | Deliverables |
|--|---|--|
| Test selected accessions in Mother and Baby trials. | Promising landraces have been identified and evaluated in <i>Kharif</i> 2008 at farmer's field through mother and baby trials and phenology. Yield and yield components related data was collected. | Mother and baby trials conducted as per protocol developed for mother and baby trials. Seeds of all the promising entries available |
| Analyze data across locations years. | Data of mother and baby trials recorded location wise | Quantitative and qualitative data of mother and baby trials being analysed. |
| Evaluation of ricebean accessions for hardseededness | Physiological study on hardseededness in landraces | 21 accessions grown in observation nursery in different production environment were evaluated |
| Field evaluation of ricebean germplasm | Study on date of planting in post rainy season and winter, on-farm /on-station Population genetics and outcrossing behavior Seed multiplication of adapted accessions | 4 best performed accessions in mother trial in 2008 evaluated for suitability in post rainy season LRGR 91 and LRGR 117 evaluated for genetics of seed colour behaviour in isolated and mixed test plots. About 500 kg of 4 adapted accessions harvested |

2.4.2. Summary of work in years 1-3

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.

In 2008, the 103 germplasm lines collected in 2006 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 were collected from different areas of the country during *rabi* (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 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.

Mother and baby trials were conducted on a large scale in the project area. The data on yield and yield attributes in mother and baby trials were recorded and statistically analyzed. Matrix ranking was done in mother trials on the basis of farmer's preference simultaneously in baby trials farmer's preference was recorded. Most farmers preferred high yielding, short duration and bold seeded varieties of ricebean because land races are of long duration type. They had given the maximum numbers in matrix ranking to those varieties having the bold seed and early in maturity.

In Nepal, the twelve best performing accessions from 2007 were evaluated in mother trials at Darbar Devasthan and Simichaur VDCs of Gulmi district; Purkot and Dulegauda VDCs of Tanahun district and Ratanchura VDC of Sindhuli district. NPGR 0076 (1555 kg/ha) and NPGR 05364 (1328 kg/ha) followed by LRGR 103 (1146 kg/ha), LRGR 111 (1116 kg/ha) and LRGR 117 (1091 kg/ha) gave the highest grain yield in Tanahun district. In Simichaur VDC grain yield of NPGR 00015 (986 kg/ha), NPGR 00076 (857 kg/ha), LRGR 111 (815 kg/ha), NPGR 00008 (800 kg/ha), NPGR 05364 (786 kg/ha) and LRGR 117 (772

kg/ha) was impressive. NPGR 06756 (1494 kg/ha) and NPGR 05364 (1140 kg/ha) performed best in terms of yield in Darbar Devisthan VDC.

The farmers preference in baby trials revealed that farmers have 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 which is suited for growing as an inter or mixed crop with maize, sorghum or on terrace risers.

Seed production was undertaken, but little seed was produced due to poor growing conditions.

To popularize ricebean cultivation among farmers, a folder on ricebean in Hindi named as (*Mungi ki Kheti*) was brought out by GVT. Two pamphlets by CSKHPAU and some publications in local languages by Assam were also prepared which contains the package and practices of ricebean. These will be useful to disseminate the project activities and enhancing the knowledge of farmers. In addition to this, 62 small-scale demonstrations of ricebean were laid out on farmer's fields in Himachal Pradesh and two in Madhya Pradesh.

2.4.3 Workpackage progress of the period

2.4.3.1 Mother and baby trials

As in previous years, a number of mother and baby trials were conducted. Five entries were tested in mother trials in each of Assam and Madhya Pradesh, and six in Himachal Pradesh. A randomised block design was used, and standard cultivation practices adopted. A total of 20 trials were held on-farm in Assam, 15 in Himachal Pradesh, and 26 in Madhya Pradesh. There was also one trial held on the University research station in Assam, and 2 in Himachal Pradesh.

Trials were evaluated through matrix ranking (Table 2.4.1). The performance of the entries was different at different locations. Results at Jorhat and Jhabua were similar. JR-1 was suitable for fodder at both these sites – at Jorhat it flowered very late. Promising entries in Assam this year were JCR-55 and JCR-51 for grain, and JR-1 for fodder. In Himachal Pradesh, RBHP-38, RBHP-41 and BRS-II were suitable for grain, and RBHP-38, and RBHP-14 for fodder, while in MP BRS-II was best for grain and JR-1 for fodder. Most farmers preferred short duration, bold seeded varieties because landraces are long duration. They gave the highest scores to varieties having these traits.

Table 2.4.1 Mean data of matrix ranking of mother trials Assam

| | Vig- our | DF | GH | Pods | D Mat | Mat | Pod Len | Seed | Seed size | Yld | Straw yld | Tot |
|-----------------------------|-------------|----|----|------|-------|-----|------------|------|--------------|-----|--------------|-----|
| Assam (Jorhat) | | | | | | | | | | | | |
| PRR-1 | 4 | 4 | 3 | 2 | 4 | 4 | 3 | 4 | 3 | 2 | - | 33 |
| JCR-51 | 5 | 3 | 4 | 3 | 3 | 3 | 4 | 3 | 4 | 5 | - | 37 |
| JR-1 | 3 | 2 | 3 | 4 | 2 | 2 | 3 | 4 | 3 | 3 | - | 29 |
| JCR-55 | 5 | 3 | 4 | 4 | 3 | 3 | 4 | 3 | 4 | 5 | - | 38 |
| BRS-II | 4 | 4 | 3 | 2 | 4 | 4 | 2 | 3 | 3 | 2 | - | 31 |
| Himachal Pradesh (Palampur) | | | | | | | | | | | | |
| RBL-6 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 33 |
| BRS-II | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 47 |
| RBHP-14 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 36 |
| RBHP-38 | 5 | 4 | 5 | 5 | 5 | 4 | 5 | 5 | 4 | 5 | 4 | 51 |
| RBHP-41 | 4 | 4 | 5 | 3 | 4 | 4 | 4 | 4 | 5 | 4 | 5 | 46 |
| RBHP-42 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 43 |

| | Vig- our | DF | GH | Pods | D Mat | Mat | Pod Len | Seed | Seed size | Yld | Straw yld | Tot |
|------------------------|-------------|----|----|------|-------|-----|------------|------|--------------|-----|--------------|-----|
| Madhya Pradesh (Jabua) | | | | | | | | | | | | |
| BRS-II | 4 | 5 | 4 | 5 | 6 | 5 | 6 | 5 | 5 | 6 | - | 51 |
| PRR-1 | 4 | 5 | 5 | 5 | 4 | 5 | 5 | 6 | 5 | 5 | - | 49 |
| JR-1 | 5 | 5 | 5 | 6 | 4 | 5 | 6 | 6 | 6 | 5 | - | 53 |
| JCR-55 | 5 | 4 | 5 | 4 | 4 | 5 | 5 | 5 | 6 | 5 | - | 48 |
| JCR-51 | 5 | 2 | 3 | - | - | - | - | - | - | - | - | 10 |

In Nepal, mother trials were conducted in five districts: Gulmi, Kavre, Khanikola, Kumaltar and Tanahun. On average, NPGR-836 and NPGR-05364 were highest yielding.

Table 2.4.2: Average performance of ricebean entries in five districts of Nepal.

| Genotype | Mean grain yield (kg/ha) |
|------------|--------------------------|
| LRGR-103 | 581.9 |
| LRGR-111 | 615.1 |
| LRGR-117 | 633.8 |
| LRGR-91 | 699.6 |
| LRGR-99 | 516.9 |
| NPGR-00008 | 562.7 |
| NPGR-00015 | 717.1 |
| NPGR-00076 | 762.1 |
| NPGR-00194 | 613.8 |
| NPGR-05364 | 814.3 |
| NPGR-05420 | 549.0 |
| NPGR-06756 | 836.9 |

Baby trials were again conducted by all partners, with either 2 or 3 entries at each location. Standard cultivation practices were adopted, as in the mother trials, and all trials were conducted on-farm. In 2009/10, JR-1, BRS-II and JCR-55 were tested in Assam, BRS-II, RBL-6 and JR-1 in MP and Gujarat, and RBHP-38, RBHP-42 and BRS-II in HP. A total of 30 trials were held in Assam and HP, and 26 in MP and Gujarat

All trials were on silty clay loam / clay loam soils, with farm yard manure applied and the crop sown after either 2 or 3 ploughings. Neither green manure nor artificial fertilizer were applied. Insecticide was used in all cases to combat attacks by pests such as blister beetles, but no herbicide use was reported, and there were no diseases noted. Farmer's perceptions were recorded, and as an example the trials in HP are shown (Table 2.4.3).

Table 2.4.3: Farmer's perceptions for ricebean varieties in baby trials in HP.

| Trait | RBHP-38 | RBHP-42 | BRS-II |
|----------------------------------|------------------|---------------|---------------|
| Flowering | Early | Early | Early |
| Pods | More | More | More |
| Pod size (small, medium, large) | Medium | Medium | Medium |
| No. of seed/pod | More | Less | Less |
| Shattering | Yes | Yes | Yes |
| Growth habit | Indeterminate | Indeterminate | Indeterminate |
| Grain type (bold, medium) | Medium | Medium | Medium |
| Maturity (early, medium, late) | Medium | Medium | Medium |
| Fodder quality (good, excellent) | Good | Good | Good |
| Average yield (g per 150 seed) | 35 kg | 25 kg | 27 kg |
| Overall liking | Very Good | Good | Good |

Farmers preferred dwarf varieties of short duration that allowed the timely sowing of a following crop of vegetables, wheat, or pulses.

2.4.3.2 Out of season planting

2.4.3.2.1 Post rainy season trials

Ricebean is a photosensitive, summer legume and the traditional practice is to sow ricebean from May-June to early July. However, the “late” (August sown) trials conducted in Nepal in 2008 showed that crop duration and vegetative growth could successfully be reduced and suggested that reasonable yields might be obtained, so giving farmers an additional option. Based on this, post-rainy season trials were conducted in four different locations from the low to the mid-hills (Table 2.4.4). Two of the four trials were coordinated by LI-BIRD (Purkot VDC, Tanahun district and Dhikurpokhari VDC, Kaski district), and two (Rampur, Chitwan district and Namdu, Dolakha district) by NARC. The trial in Namdu (high altitude, 900 m) failed due to heavy rainfall and pest infestation and is not reported, some plants survived. The four best performing accessions from the mother trials (2008); LRGR 91 (Dang), LRGR 111 (Gulmi), NPGR 05364 (Bhojpur), and NPGR 00008 (Nuwakot) were included and evaluated for yield traits. Sowing dates were 15 days apart (1, 15 and 30 August in Tanahun and Chitwan), and 3 and 18 August, and 1 September in Kaski).

Details of plots, methodology and observations undertaken and the sites are given in Annex 5. The plots were managed according to customary farmers’ practices, and neither fertilizer nor irrigation was applied.

Table 2.4.4. Locations of post rainy season trials

| District | Site | Altitude | Organization |
|----------|---------------------|----------|--------------|
| Chitwan | Rampur/Sharadanagar | 200 | NGLRP/NARC |
| Tanahun | Purkot | 566 | LI-BIRD |
| Kaski | Dhikurpokhari | 1226 | LI-BIRD |
| Dolakha | Kabre | 1400 | HCRP/NARC |

The trial conducted by NARC in Dolakha was a failure. The research plots were heavily affected by pod borer, aphid and blister beetle. The data generated was not analyzable. However, surviving plants of NPGR 05364 and LRGR 111 were better than the other two accessions.

Table 2.4.5. Mean of days to maturity for four ricebean accessions tested in three different locations, 2009

| Location | Date of sowing | LRGR 91 | NPGR 05364 | LRGR 111 | NPGR 00008 | Mean |
|-------------|----------------|--------------|--------------|--------------|--------------|--------------|
| Chitwan | 1 August | 98.5 | 98.5 | 98.5 | 98.5 | 98.5 |
| | 15 August | 114.5 | 114.5 | 114.5 | 114.5 | 114.5 |
| | 30 August | 119.0 | 119.0 | 119.0 | 119.0 | 119.0 |
| Mean | | 110.7 | 110.7 | 110.7 | 110.7 | 110.7 |
| Tanahun | 1 August | 107.3 | 114.8 | 104.3 | 106.0 | 108.1 |
| | 15 August | 102.8 | 111.3 | 101.8 | 101.8 | 104.4 |
| | 30 August | 103.3 | 114.0 | 99.0 | 102.8 | 104.8 |
| Mean | | 104.4 | 113.3 | 101.7 | 103.5 | 105.7 |
| Kaski | 1 August | 116.8 | 126.8 | 115.0 | 118.8 | 119.3 |
| | 15 August | 119.3 | 126.8 | 111.8 | 119.0 | 119.2 |
| | 30 August | 115.5 | 127.5 | 112.0 | 119.0 | 118.5 |
| Mean | | 117.2 | 127.0 | 112.9 | 118.9 | 119.0 |

The trials conducted by NARC through National Grain Legume Research Programme performed better in terms of vegetative growth as well as yield. However, the trial did not perform as per the expectation of the research. Significant reduction in vegetative growth was anticipated. The result showed that at the lower altitude, even the late sown ricebean can give an equally high yield as ricebean sown in the

conventional season/time. But the most important issue for ricebean in the lower altitude and basically the productive and commercial agriculture areas ricebean is hard to be adjusted in the cropping pattern. Thus, the best option for these areas would be to grow ricebean in home gardens for family consumption.

The result showed that maturity period has been reduced in post rainy season crop. The mean crop duration ranged from 98 days to 119 days (Table 2.4.5). In Chitwan, crops sown on 1 August matured much earlier than on the other two dates. In Tanahun, mean days to maturity appeared to be similar while mean days to maturity appeared slightly more in case of Kaski.

Table 2.4.6. Mean plant height (cm) for four ricebean accessions tested in three different locations, 2009

| Location | Date of sowing | LRGR 91 | NPGR 05364 | LRGR 111 | NPGR 00008 | Mean |
|-------------|----------------|--------------|--------------|--------------|--------------|--------------|
| Chitwan | 1 August | 201.8 | 204.8 | 206.5 | 202.0 | 203.8 |
| | 15 August | 178.0 | 176.0 | 173.0 | 167.5 | 173.6 |
| | 30 August | 116.8 | 146.8 | 133.3 | 143.0 | 134.9 |
| Mean | | 165.5 | 175.8 | 170.9 | 170.8 | 170.8 |
| Tanahun | 1 August | 63.5 | 55.7 | 56.2 | 64.5 | 60.0 |
| | 15 August | 49.0 | 50.1 | 50.0 | 56.2 | 51.3 |
| | 30 August | 54.4 | 50.9 | 49.8 | 49.7 | 51.2 |
| Mean | | 55.6 | 52.2 | 52.0 | 56.8 | 54.1 |
| Kaski | 1 August | 43.2 | 55.6 | 42.6 | 42.8 | 46.0 |
| | 15 August | 54.4 | 46.9 | 57.7 | 49.1 | 52.0 |
| | 30 August | 62.3 | 58.7 | 50.2 | 46.6 | 54.4 |
| Mean | | 53.3 | 53.7 | 50.1 | 46.1 | 50.8 |

One of the major targets of this experiment was to reduce the vegetative growth of ricebean. The result showed that there was reduced vegetative growth in all the trials in all the three locations but the growth was highly checked in the trials conducted in Tanahun and Kaski (Table 2.4.6). The result in Kaski and Tanahun was as expected in the experiment and this has given some scope of growing ricebean as a sole crop similar to black gram. A clear trend in reduced vegetative growth was observed for date of sowing in Chitwan but not in the trials in Tanahun and Kaski districts.

Table 2.4.7. Mean of grain yield (kg ha⁻¹) for four ricebean accessions tested in three different locations, 2009

| Location | Date of sowing | LRGR 91 | NPGR 05364 | LRGR 111 | NPGR 00008 | Mean |
|-------------|----------------|-------------|-------------|-------------|-------------|-------------|
| Chitwan | 1 August | 1561 | 1744 | 1281 | 1489 | 1519 |
| | 15 August | 1744 | 1646 | 1576 | 1317 | 1571 |
| | 30 August | 1462 | 1426 | 1575 | 1767 | 1557 |
| Mean | | 1589 | 1605 | 1477 | 1524 | 1549 |
| Tanahun | 1 August | 328 | 354 | 300 | 406 | 347 |
| | 15 August | 228 | 239 | 257 | 313 | 259 |
| | 30 August | 228 | 222 | 305 | 343 | 275 |
| Mean | | 262 | 272 | 287 | 354 | 294 |
| Kaski | 1 August | 233 | 336 | 100 | 232 | 225 |
| | 15 August | 320 | 288 | 335 | 286 | 307 |
| | 30 August | 347 | 460 | 398 | 347 | 388 |
| Mean | | 300 | 361 | 278 | 288 | 307 |

The mean yield of the four ricebean accessions in Chitwan clearly show that the crop can be taken in the post rainy season and in a short duration of time without much reduction in yield of grain (Table 2.4.7).

There was a suggestion that LRGR 91 and NPGR 00008 gave higher yields from the first two plantings, whereas the second and third were better for LRGR 111 and NPGR 05364. However, in both Tanahun and Kaski the yield was low as vegetative growth was highly reduced. But it was evident that there is scope for increasing yield by reducing the spacing between plants i.e. by increasing plant population, plant density. The proposed spacing was too large for the vegetative growth obtained in the experiment. No clear trend in yield was observed for date of sowing in all the trials conducted in Chitwan, Tanahun and Kaski districts.

Importance of ricebean from the view point of fodder cannot be overlooked. The overall biomass was reduced in both the trials in Tanahun and Kaski (Table 2.4.8). This could be managed by maintaining a proper spacing and plant population. However, the result did not show a proper trend of reduction of biomass for date of sowing.

Table 2.4.8. Mean biomass for four ricebean accessions tested in three different locations, 2009

| Location | Date of sowing | LRGR 91 | NPGR 05364 | LRGR 111 | NPGR 00008 | Mean |
|----------|----------------|---------------|---------------|---------------|---------------|---------------|
| Chitwan | 1 August | NA | NA | NA | NA | NA |
| | 15 August | NA | NA | NA | NA | NA |
| | 30 August | NA | NA | NA | NA | NA |
| | Mean | NA | NA | NA | NA | NA |
| Tanahun | 1 August | 1407.5 | 2047.9 | 1220.4 | 1883.3 | 1639.8 |
| | 15 August | 769.2 | 1191.7 | 858.3 | 1039.6 | 964.7 |
| | 30 August | 865.8 | 960.4 | 1189.2 | 1047.1 | 1015.6 |
| | Mean | 1014.2 | 1400.0 | 1089.3 | 1323.3 | 1206.7 |
| Kaski | 1 August | 689.2 | 1205.4 | 412.1 | 621.7 | 732.1 |
| | 15 August | 1626.7 | 1131.3 | 989.6 | 1035.0 | 1195.6 |
| | 30 August | 1317.1 | 1764.2 | 1260.0 | 1161.3 | 1375.6 |
| | Mean | 1211.0 | 1366.9 | 887.2 | 939.3 | 1101.1 |

2.4.3.2.2 Winter-season trials

Experience from field visits and knowledge shared among different people and FOSRIN partners, suggested the rare possibility of ricebean cultivation during the winter season. In Nepal, an adaptation trial for ricebean in winter season was designed and conducted in Jhapa and Dang districts, Nepal. The trial in Dang was a failure. However, the trial in Jhapa gave some exciting results. Biomass and days to maturity decreased significantly. Some accessions showed some promise for yield with good pod formation. Thus, the study showed that ricebean could be one of the potential winter legumes which could be utilized in rainfed *rabi* cropping areas especially rice fallows. Based on the experiences from previous years' trial in Jhapa, two different trials were designed for 2009/10 in Nepal, and one in India. In India, a trial was carried out in two villages, Sarmariya and Karamba, in Dahod district, Gujarat, using two genotypes: BRS-II and RBL-6 (Table 2.4.9).

Table 2.4.9: Details of winter ricebean entries tested in Dahod district of Gujarat in 2009.

| Farmer | Entry | Date sowing | Yield (kg/h) | Seedling vigour* | Days to 50% flowering | Pods / plant | Days to maturity | Pod length (cm) | Seed / pod | 100 seed weight (g) | Height (cm) |
|---------------------------|--------|-------------|--------------|------------------|-----------------------|--------------|------------------|-----------------|------------|---------------------|-------------|
| Barsingh FataBhai Kamol | RBL-6 | 14/11 | 242.8 | F | 70 | 28 | 110 | 5.9 | 5 | 6 | 80 |
| | BRS-II | | 275.0 | M | 75 | 30 | 115 | 6.4 | 6 | 5 | 74 |
| Dangi Amrootbhai Samabhai | RBL-6 | 15/11 | 239.1 | F | 70 | 28 | 110 | 5.9 | 5 | 6 | 80 |
| | BRS-II | | 254.2 | M | 75 | 30 | 115 | 6.4 | 6 | 5 | 74 |

*F=fast, M=medium

All plants were of semi-erect growth habit and continuous maturity type. It was observed in this set of test that these two entries do set the seed but poor grain yield. These may be suitable for further confirming their suitability for off season and their insensitivity to temperatures and photoperiods.

In the adaptation trial, in Nepal, the objective was to identify the accessions which can thrive well in the winter season. The best performing accessions in the observation nurseries, 2008, and the best performing lines in the mother trials, 2008 and winter adaptation trials, 2008/09 were tested (Annex 6). The first sowing was in the first week, and the second in the third week, of November, 2009. This trial showed that not all the accessions perform uniformly in winter season (Table 2.4.10). Accessions differed significantly for days to flowering, days to maturity, number of pods per plant, grain yield and biomass yield. Days to maturity was not reduced much compared to main season crop. Yield was impressive with LRGR 44 giving yield more than 2 tonnes per ha. NPGR 05372 was late compared to others while NPGR 05364 was the earliest. Biomass yield in NPGR 05372 and LRGR 129 was high but the grain yield was very poor. Thus, based on yield LRGR 44, NPGR 05422, NPGR 05364 were the best performers.

Testing response to seed priming and de-topping. In winter, germination is one of the major problems for ricebean. Similarly, staking is another problem. The two best performing accessions of the winter adaptation trials in 2008/09, LRGR 103 & LRGR 111 was used (Annexe 7). The accessions were tested for their response to de-topping and seed priming. Results showed that ricebean could be one an option for winter fallows, but wider testing is required for further validation. Most ricebean accessions are indeterminate in growth habit, but those tested in winter 2008/09 showed a tendency to a determinate habit, with long tendrils of the tip of the plants were prominent. This part appeared to be useless from yield point of view. Several papers indicated that priming promotes better vegetative growth and improvement in yield. The result obtained from the experiment did not show any significant advantage to priming and de-topping in winter ricebean trials, with significant differences between treatments only in pod length (Table 2.4.11).

Table 2.4.10: Mean results of agronomic traits in winter adaptation trials, Jhapa, 2009/10

| Genotype | Days to flowering | Days to Maturity | Pods per plant | Pod length (cm) | Grain yield (t ha ⁻¹) | Biomass yield (kg plot ⁻¹) |
|-------------|-------------------|------------------|----------------|-----------------|-----------------------------------|--|
| NPGR 06591 | 88.0 | 150 | 30.7 | 7.67 | 1.079 | 2.93 |
| NPGR 05372 | 100.0 | 152 | 34.0 | 7.33 | 0.546 | 3.73 |
| NPGR 05422 | 89.0 | 139 | 56.3 | 8.07 | 1.897 | 2.15 |
| NPGR 05410 | 83.7 | 138 | 47.0 | 7.83 | 1.423 | 2.00 |
| LRGR 129 | 100.0 | 151 | 33.0 | 7.43 | 0.578 | 3.60 |
| LRGR 124 | 87.0 | 150 | 33.0 | 7.80 | 0.926 | 3.32 |
| LRGR 44 | 100.3 | 145 | 51.3 | 8.53 | 2.066 | 3.57 |
| LRGR 117 | 100.3 | 152 | 33.3 | 7.90 | 1.203 | 3.37 |
| NPGR 05420 | 103.3 | 142 | 40.0 | 7.37 | 1.282 | 1.18 |
| LRGR 91 | 89.0 | 146 | 42.3 | 7.87 | 1.063 | 2.98 |
| NPGR 00008 | 86.0 | 135 | 50.0 | 6.93 | 1.219 | 1.43 |
| NPGR 05364 | 84.0 | 133 | 60.7 | 7.67 | 1.666 | 1.25 |
| Mean | 92.6 | 144 | 42.6 | 7.70 | 1.246 | 2.63 |
| LSD | 7.25** | 5.0** | 18.80* | -ns | 0.651** | 1.264** |

2.4.3.3 Hardseededness

21 accessions of ricebean from the observation nursery (OBN) were evaluated for hard seededness of fresh seeds produced in Kabre (high-hill), Khumaltar (mid-hill) and Rampur (lowland) conditions in 2008. These comprised of NPGR and LRGR collections from 19 districts of Nepal and were of different seed coat colour. Seeds of OBNs from Rampur, Kabre and Khumaltar were procured and altogether 63 samples (21 accessions x 3 production environment) were used to understand hardseededness in relation to field establishment and kitchen values. The experimental design was of randomized complete block

carried out in the Seed Research Laboratory, NARC, at Khumaltar. 100 seeds of each accession were soaked in water for overnight and then plated 25 seeds each in 4 replications between moistened germination papers at room temperature and observed for sprouting and hard seeds (seeds not absorbing water). The protocol is described in Annex 8.

Table 2.4.11: Mean results of agronomic traits in winter adaptation trials, Jhapa, 2009/010

| Trt | Days to flowering | Days to maturity | Height (cm) | Pod length (cm) | Grain yld (t ha ⁻¹) | Biomass yld (kg plot ⁻¹) |
|---------------------------------------|-------------------|------------------|-------------|-----------------|---------------------------------|--------------------------------------|
| LRGR103 + dry seed + no de-topping | 130.3 | 159.0 | 27.3 | 6.90 | 0.88 | 1833 |
| LRGR103 + dry seed + de-topping | 130.3 | 157.0 | 25.0 | 7.17 | 0.63 | 1583 |
| LRGR103 + primed seed + no de-topping | 131.0 | 157.7 | 23.7 | 7.80 | 0.62 | 1633 |
| LRGR103 + primed seed + de-topping | 130.7 | 156.3 | 22.3 | 7.03 | 0.53 | 3883 |
| LRGR111 + dry seed + no de-topping | 131.7 | 160.0 | 25.3 | 6.20 | 0.51 | 1900 |
| LRGR111 + dry seed + de-topping | 131.3 | 159.7 | 29.0 | 6.23 | 1.15 | 2067 |
| LRGR111 + primed seed + no de-topping | 130.7 | 159.9 | 25.3 | 6.57 | 0.49 | 1700 |
| LRGR111 + primed seed + de-topping | 130.7 | 157.7 | 25.3 | 6.53 | 0.61 | 2000 |
| Mean | 130.8 | 158.40 | 25.4 | 6.80 | 0.68 | 2075 |
| LSD | -ns | -ns | -ns | 0.676* | -ns | -ns |

There were differences in development and occurrence of hard seeds. The percentage of hard seeds ranged from 0 to 69. Two genotypes, NPGR 00184 (69%) and LRGR 133 (45%), had the highest levels of hard seeds for the seeds produced in Khumaltar and Rampur. However, the proportion of hard seeds produced in Kabre was comparatively low, with the highest (20%) being in NPGR 05374, a collection from Gorkha (high-hill). These genotypes represented the high-hill, mid-hill and lowland agroecosystems and they also vary in seed coat colour (Table 2.4.12). The amount of hardseededness was irrespective of seed coat colour, and is not associated with either production environment or genotype.

Table 2.4.12: Hardseededness in ricebean genotypes observation nurseries in three production environments in 2009

| Accession | District of origin | Kabre (high-hill) | Rampur (lowland) | Khumaltar (mid-hill) | Colour |
|------------|--------------------|-------------------|------------------|----------------------|----------------|
| NPGR 00184 | Kavre | 3 | 23 | 69 | black |
| NPGR 00005 | Nuwakot | 2 | 14 | 24 | yellow/mottled |
| LRGR 123 | Palpa | 8 | 39 | 15 | mottled |
| LRGR 133 | Palpa | 0 | 45 | 43 | yellow |
| LRGR 139 | Gulmi | 3 | 30 | 8 | yellow |
| NPGR 05381 | Lamjung | 4 | 20 | 30 | yellow |
| NPGR 05383 | Tanahu | na | 5 | 18 | yellow |
| NPGR 05424 | Dhankuta | na | 13 | 20 | red/yellow |
| NPGR 05422 | Dhankuta | na | 14 | 13 | red/yellow |
| NPGR 00191 | Dang | na | 15 | 15 | red |
| LRGR 113 | Gulmi | 2 | 5 | 6 | yellow |
| LRGR 87 | Dang | 0 | 8 | 39 | yellow |
| NPGR 05410 | Dhankuta | 4 | 0 | 9 | yellow/maroon |
| LRGR 153 | Kavre | 2 | 19 | 22 | yellow |
| LRGR 143 | Kaski | 3 | 25 | 30 | yellow/maroon |
| NPGR 00189 | Kavre | | 3 | 18 | black |
| NPGR 05372 | Gorkha | 7 | 13 | 9 | yellow |
| NPGR 05374 | Gorkha | 20 | 11 | 29 | yellow |
| NPGR 08381 | Myagdi | 11 | 16 | 16 | mottled |
| NPGR 05367 | Bhojpur | 1 | 1 | 0 | yellow |
| NPGR 05425 | Dhankuta | na | 33 | 27 | yellow |

na = not available (not tested)

2.4.3.4 Population genetics and outcrossing

Assessment of the genetic behaviour of seed colour traits in Nepalese ricebean germplasm was carried out on-farms and stations in different agro-environments in 2009. The best performing and adapted genotypes in mother trials in 2008 were included in these studies to evaluate their additional potential and suitability for cultivation in fallow lands matching *ghaiya* rice or maize-based cropping pattern in the post-rainy season and to understand the genetics of seed coat colour inheritance.

There are some indications from the literature that ricebean has a degree of outcrossing behaviour, backed up by earlier observations within FOSRIN. The appearance of mixed colour ricebean grains from a single colour seed sown suggested this, and so a detailed genetic study is necessary to identify the nature of this trait. Initially, breeding was carried out to purify the population. For this, four good performing accessions were selected for improvement through population breeding technique. Among the 12 accessions in the 2008 mother trial, LRGR 42, LRGR 103, LRGR 111 and LRGR 117 were selected by LI-BIRD based on colour, size of seed and yield potential. Five superior plants from each of these were selected and harvested individually, and progeny rows of them grown. The trials were maintained in proper isolation to avoid any outcrossing. The summary of the breeding procedure is in Annex 9.

A total of 250 plants were maintained for each accession including five progeny rows. The best performing plants from each progeny row of the different accessions were selected (Table 2.4.13), based on plant type, pod formation, and disease and pest resistance.

Table 2.4.13: Plants selected for further improvement through population breeding, Gulmi, 2009/10

| Accession | Progeny | | | | | Total |
|-----------|---------|----|----|----|----|-------|
| | 1 | 2 | 3 | 4 | 5 | |
| LRGR 42 | 12 | 17 | 3 | 2 | 0 | 34 |
| LRGR 103 | 22 | 25 | 21 | 29 | 24 | 121 |
| LRGR 111 | 11 | 14 | 12 | 18 | 8 | 64 |
| LRGR 117 | 21 | 19 | 21 | 22 | 16 | 99 |

At NARC, two sets, each of two accessions (dominant and recessive traits for seed coat colour) were studied for outcrossing behaviour. LRGR 111 (dominant – maroon seed coat) and LRGR 91 (recessive – creamish yellow seed coat) were the two best performing and adapted genotypes selected. This activity was carried out at the Rampur on-station of the National Grain Legume Research Programme (NGLRP). The trial was designed as 40 plants grown in isolation as pure line plots, as well as mixed plots of equal populations of these genotypes in alternate rows spaced at 120 x 75 cm (Table 2.4.14). A similar trial was carried out with NPGR 00184 (black and bold seed) and LRGR 44 (creamish yellow and bold seed). The protocol in detail is attached in Annex 9.

Table 2.4.14: Planting details and seed coat colour of accessions used in the experiment

| Genotypes | Area (m ²) | Spacing (cm) | Seed colour | Gene |
|-----------------------------|------------------------|--------------|------------------------|-----------|
| SET A (sown July 6): | | | | |
| LRGR 111 | 600 | 120 x 75 | Maroon | Dominant |
| LRGR 91 | 600 | 120 x 75 | Creamish yellow | Recessive |
| LRGR 111 + LRGR 91 | 672 | 120 x 75 | Both in alternate rows | |
| LGR 44 | 75 | 120 x 75 | Creamish yellow | Recessive |
| NPGR 00184 | 75 | 120 x 75 | Black | Dominant |
| LGR 44+ NPGR 00184 | 150 | 120 x 75 | Both in alternate rows | |

The yield and yield components are summarised in Table 2.4.15. The first set (LRGE 91 and LRGR 111) matured about 10 days earlier than the second set (LRGR 44 and NPGR 00184), as the former were planted early and the soil was a loam of low water holding capacity. The early planted accessions were tallest, while the shortest (about 1.5 m) was LRGR 44 (Table 5). LRGR 44 and NPGR 00184 produced mean grain yields

of 110 g/plant, while LRGR had the lowest yield at 50 g/plant. The number of seeds per pod was more related to grain yield than to the number of pods per plant.

Table 2.4.15: Yield and yield components of accessions (n=40) at Rampur, 2009

| Accession | Days to harvest | Total pods | Seeds per 10 pods | Empty pods | Seed yield (g/plant) | Plant height (cm) | Primary branches |
|------------|-----------------|------------|-------------------|------------|----------------------|-------------------|------------------|
| LRGR 91 | 135-150 | 293 | 58 | 6 | 95 | 243 | 6 |
| LRGR 111 | 142-149 | 166 | 58 | 1 | 51 | 270 | 11 |
| LRGR 44 | 160 | 147 | 71 | 3 | 110 | 218 | 8 |
| NPGR 00184 | 162 | 220 | 82 | 7 | 109 | 152 | 11 |

In the pure plots of recessive genotypes, we harvested creamish yellow seeds (the original colour) (89.7%), brown (6.7%), green mottled (3.0%) and black (0.6%) in LRGR 91, and creamish yellow (96%) and maroon (4%) in LRGR 44 (Table 6). Similarly in the pure plots of dominant genotypes, LRGR 111 (maroon) showed segregation into maroon (81.6%), creamish yellow (14.4%), green mottled (2.5%), brown (1.3%) and black (0.3%), while black seeded genotype (NPGR 00184) segregated into black (88%), creamish yellow (9%) and maroon (3%) (Table 2.4.16).

Table 2.4.16: Four accessions showing segregation in seed coat colour when sown in isolation at Rampur

| Accession | Seed colour at sowing | Seed colour at harvest | | | | | Total |
|------------|-----------------------|------------------------|-------|---------------|--------|-------|-------|
| | | Yellow | Brown | Green Mottled | Maroon | Black | |
| LRGR 91 | Yellow | 602 | 45 | 20 | 4 | 0 | 671 |
| LRGR 111 | Maroon | 110 | 10 | 19 | 624 | 2 | 765 |
| LRGR 44 | Cream | 90 | 0 | 0 | 4 | 0 | 94 |
| NPGR 00184 | Black | 9 | 0 | 0 | 3 | 85 | 97 |

However, in the mixed plots, the proportions were maroon (85.7%), creamish yellow (11.0%), green mottled (2.6%) and brown (0.7%) in LRGR 91 x LRGR 111, and for LRGR 44 x NPGR 00184 black (54.5%), creamish yellow (43.4%) and maroon (2.1%) (Table 2.4.17).

Table 2.4.17: Four accessions showing segregation in seed coat colour when sown in isolation at Rampur

| Accession | Seed colour at harvest | | | | | Total |
|----------------------|------------------------|-------|---------------|--------|-------|-------|
| | Yellow | Brown | Green Mottled | Maroon | Black | |
| LRGR 91 x LRGR 111 | 30 | 2 | 7 | 233 | 0 | 272 |
| LRGR 44 x NPGR 00184 | 43 | 0 | 0 | 2 | 54 | 99 |

2.4.3.5 Seed multiplication of adapted genotypes

Five good performing, well-adapted genotypes: LRGR 91, LRGR 111, NPGR 00008, NPGR 00076 and NPGR 05364 were multiplied at NARC research stations to produce sufficient seeds for dissemination in 2010 under a scaling up programme (Table 2.4.18).

Table 2.4.18: Promising genotypes under seed multiplication and their planting details

| Location | Accession | Area (m ²) | Sowing date | Spacing (cm) |
|----------------|------------|------------------------|--------------|--------------|
| NGLRP, Rampur | LRGR 111 | 600 | 06 July 2009 | 120 x 75 |
| | LRGR 91 | | 15 July 2009 | 120 x 75 |
| NORP, Nawalpur | NPGR 00076 | 620 | 09 July 2009 | 75 x 50 |
| | NPGR 05364 | | 10 July 2009 | 75 x 50 |
| HCRP, Kabre | LRGR 91 | 1596 | 08 July 2009 | 100 x 75 |
| | LRGR 111 | | 09 July 2009 | 100 x 75 |
| ABD, Khumaltar | NPGR 05364 | 1380 | 05 June 2009 | 100 x 75 |
| | NPGR 00076 | | 06 June 2009 | 100 x 75 |

The following quantity of seeds was harvested, and is available for dissemination and production in 2010/11 (Table 2.4.19).

Table 2.4.19: Seed harvested (kg) from multiplication plots, 2009

| Accessions | Kabre | Khumaltar | Rampur | Nawalpur | Itahari | Total | Seed colour |
|--------------|-----------|------------|-----------|-----------|-----------|------------|-------------|
| NPGR 00008 | - | 120 | - | - | - | 120 | Cream |
| NPGR 00076 | - | 120 | - | 15 | - | 135 | Mottled |
| NPGR 05364 | - | - | - | - | 70 | 70 | Cream |
| LRGR 91 | 60 | - | 32 | - | - | 102 | Cream |
| LRGR 111 | 29 | - | 55 | - | - | 85 | Maroon |
| Total | 89 | 240 | 87 | 15 | 70 | 512 | |

In Nepal, seed production was initiated by LI-BIRD at the community level in Tanahun, Gulmi, Palpa and Dang district. This is started in a very small scale as there was no sufficient source seed available. Similarly, NARC also produced seeds of different accessions in different research stations. Ricebean seed was produced in NGLRP, ABD, HCRP, NORP and NJRP. A total of 85 kg was produced by LI-BIRD and 578 kg by NARC in 2009 (Table 2.4.20).

Table 2.4.20. Seed produced by LI-BIRD and NARC in 2009

| Accessions | NGLRP | ABD | HCRP | LI-BIRD | NORP | NJRP | Total |
|--------------|------------|------------|-----------|-----------|-----------|-----------|------------|
| LRGR 42 | | | | 1 | | | 1 |
| LRGR 44 | 9 | | | | | | 9 |
| LRGR 91 | 32 | | 60 | 10 | | | 102 |
| LRGR 103 | | | | 30 | | | 30 |
| LRGR 111 | 55 | | 29 | 15 | | | 99 |
| LRGR 117 | | | | 15 | | | 15 |
| NPGR 00008 | | 180 | | 4 | | | 184 |
| NPGR 00076 | | 120 | | | 15 | | 135 |
| NPGR 00184 | 8 | | | | | | 8 |
| NPGR 05364 | | | | 10 | | 70 | 80 |
| TOTAL | 104 | 300 | 89 | 85 | 15 | 70 | 663 |

Similarly in India, CSKHPKV produced more than 100 kg of ricebean seed of three varieties in 2009 for distribution as IRD in the coming ricebean season (Table 2.4.21)

Table 2.4.21. Seed production in Himanchal Pradesh, India, 2009

| Accession | Location | Amount | Responsible organization |
|-----------|----------|--------|--------------------------|
| BRS-II | Palampur | 70kg | CSK HPKV Palampur |
| Nainy | Palampur | 30 kg | CSK HPKV Palampur |
| RBHP-38 | Palampur | 5 kg | CSK HPKV Palampur |

Informal research and development (IRD) is a very useful means of disseminating information on any crop. A total of 130 packets ricebean seed were distributed this way to 130 farmers in Tanahun, Kaski, Syangja and Gulmi districts by LI-BIRD (Table 2.4.221). Farmers in Alamdevi VDC, Syangja requested seed of different accessions for the coming season as the IRD performed excellent in the farmers' field.

Similarly, a total of 118 packets were distributed through IRD to farmers in Chepang (one of the indigenous tribal communities of Nepal) in Bhumlichowk and Ghyalchowk VDCs of Gorkha district, and Kholagaun and Rasoli VDCs of Tanahun district through another LI-BIRD project '*Land use transition and human health in Eastern Himalayas: An adaptive ecosystem approach*' (Table 2.4.23).

The project has reported that most of the Chepang farmers have saved the seed for the next season and many farmers have requested more seeds.

Table 2.4.22. Details of ricebean IRD distribution, 2009

| District | Total farmers | IRD distributed site(s) | Distributed ricebean accessions |
|--------------|---------------|---|---|
| Tanahun | 19 | Manapang VDC | NPGR 00015, NPGR 00194, LRGR 75, LRGR 117 and LRGR 42 |
| Kaski | 43 | Lumle, Sallyan, Dikurpokhari, Dhampus and Sarangkot VDC | LRGR 42, LRGR 75, NPGR 00015, NPGR 00194, NPGR 05420 and NPGR 00195 |
| Syangja | 12 | Aalamdevi VDC | LRGR 103, LRGR 15, NPGR 05420 and LRGR 117 |
| Gulmi | 56 | Simichaur VDC | LRGR 91, LRGR 111, LRGR 99, LRGR 103, NPGR 00008, NPGR 00015, NPGR 00076, NPGR 00194, NPGR 05364, NPGR 05420 and NPGR 06756 |
| Total | 130 | | |

Table 2.4.23. Distribution of IRD to Chepang farmers, 2009

| District | Total farmers | IRD distributed site(s) | Distributed ricebean accessions |
|--------------|---------------|-------------------------------|---|
| Gorkha | | Bhumlichok and Ghyalchok VDCs | NPGR 00184, LRGR 130, LRGR 106, LRGR 139, LRGR 143, LRGR 35 and LRGR 124. |
| Tanahun | | Kholagaun and Rasoli VDCs | NPGR 00184, LRGR 130, LRGR 106, LRGR 139, LRGR 143, LRGR 35 and LRGR 124. |
| Total | 118 | | |

2.4.3.6 Comparison of ricebean and blackgram

Blackgram (*Vigna mungo*) is the major summer legume in marginal areas in the mid hills of Nepal, but it is not a flexible crop, and leaves the farmers few options in terms of how to grow it as it has a long growing season. As ricebean is adapted to marginal conditions, and given its possibilities as a post-rainy season crop, this trial was designed to compare the performance of ricebean post-rainy season with blackgram. The trial was conducted by LI-BIRD in Dulegaunda-3, Devasthan in Tanahun district. Four ricebean accessions: LRGR 91, LRGR 111, NPGR 05364 and NPGR 00008 were tested along with three blackgram genotypes. Two of these, BLG 0069-1 and BLG 003-2-1, are promising genotypes provided by the NGLRP, Chitwan and one is a local variety. The details of plots, methodology, observations and the site are given in Annex 10. The plots were managed according to customary farmers' practices, and neither fertilizer nor irrigation were applied.

Table 2.4.24. Mean results of agronomic traits in post rainy season trial, Tanahun, 2009/10

| Genotype | Days to flowering | Days to maturity | Height (cm) | Grain yield (g/60 plants) | 100 grain weight (g) |
|-------------|-------------------|------------------|----------------|---------------------------|----------------------|
| NPGR 00008 | 46.3 | 84.7 | 51.8 | 0.274 | 5.97 |
| NPGR 05364 | 53.0 | 88.0 | 56.5 | 0.326 | 6.43 |
| LRGR 91 | 54.3 | 93.0 | 62.1 | 0.213 | 8.37 |
| LRGR 111 | 58.0 | 95.3 | 54.6 | 0.097 | 6.83 |
| BLG-069-1 | 37.0 | 69.0 | 17.7 | 0.051 | 4.27 |
| BLG-003-2-1 | 37.7 | 69.7 | 21.2 | 0.051 | 4.43 |
| Local | 39.7 | 73.3 | 24.7 | 0.134 | 4.20 |
| Mean | 46.6 | 81.9 | 41.2 | 0.164 | 5.79 |
| LSD | 1.93** | 2.04** | 17.75** | 0.1619* | 1.025** |

Ricebean and blackgram varieties differed significantly in days to 50% flowering, days to maturity, height, grain yield and hundred grain weight (Table 2.4.24). The pipeline black gram varieties were earlier than the local blackgram, and than ricebean, but were short, and had low yield and hundred grain weight. Three ricebean varieties (NPGR 00008, NPGR 05364 and LRGR 91) yielded more than 3 times the blackgram, and were taller, an indication of increased total biomass production. However, ricebean

was slower to mature than blackgram. The results indicate that ricebean could be a better option than blackgram in the mid hills where no crop can be taken after harvesting blackgram.

Deviations from the workplan

There were no “downward” deviations from the agreed revised workplan in this period. However, all Asian partners put significantly increased efforts into this particular WP as it will produce many unforeseen and innovative results that will be extremely valuable for farmers. Work was carried out on crossing, hardseededness, out of season cropping, and comparisons with blackgram, which was not initially foreseen, due to the importance of these issues becoming apparent as the project progressed.

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 years 1-3

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.

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 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 were formatted for the WorldFood2 standard programme.

With the aim of analysis of the specific nutritional value of ricebean, a full range of nutrient figures was also compiled in WorldFood2 format. Most values were compiled from existing literature and, due to geographic variation in the sources, and the variability of ricebean, were 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*, *pakorras* 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.

In year 3, work continued on establishing the current knowledge on the nutritive value of ricebean, and its present and potential role in human nutrition and food security at local level. The values on nutritive values found in the literature are generally from a small number of samples and varieties, and it is likely that there will be substantial variations between different varieties, and between crops grown under various conditions, but there appears to quite consistent trends in the results presented by various authors and analyses.

Some important general observations appears to be

- a very favourable amino acid composition for human consumption
- a substantial content of B vitamins and some minerals
- the absence of toxic and allergenic substances

This last point is of importance since many other pulses may have a substantial content of for instance cyanides, allergenic compounds or enzyme inhibitors that need special concern in breeding work and nutritional extension work. Ricebean is not completely free such compounds, but the evidence suggests that there are no specific risk problems with ricebean as a source of food for humans. The content of flatulence producing factors in ricebean is similar to that in similar pulses. However, mild gastritis is noted in connection with consumption of ricebean. The effects of these factors are affected by cooking methods, and the use of overnight soaking before boiling as a *dal* should be encouraged in extension work, also since soaking increases the bioavailability of several nutrients.

The reports on nutrient content (Deliverable 5.2) and recipes and food preparation (Deliverable 5.1) were submitted.

Further work links nutrient content to the actual nutritional status of various groups in the project areas. Two surveys were carried out in each of India and Nepal. Each survey team collected dietary recalls three times from 200 individuals (adult women), a total of 20,000 records. This was processed using the WorldFood2 package, using the Indian food tables for calculating the contribution of each record for 47 nutrient variables, with statistical analysis using STATA and SPSS.

The dietary surveys gave a rich source of information concerning differences in diets between the sites. Lentil is the most common pulse consumed overall, followed by field bean. Chickpea is very commonly consumed in the predominantly vegetarian Himachal Pradesh where it serves as a second pulse per day. Ricebean was the fifth most important pulse item, most commonly consumed in Himachal Pradesh.

A major difference between sites was the composition of staple grains. In Assam, rice was the main staple. In Nepal, rice was also a main staple, but maize and wheat are taken as alternative grain staples. In Himachal Pradesh, women take rice as well as wheat in most days.

Pulses are in general an important source of protein, and ricebean has a particularly good composition of essential amino acids. However, while the pulses made a considerable contribution to protein supply, protein and essential amino acids were not a nutritional risk factor in the populations, due to relatively varied other sources of protein, either in terms of milk products (Himalayan sites) or fish and pork (Assam). As a source of vitamins, the pulses are primarily important with respect to B vitamins, and especially B1 and B9 (folic acid).

The calculated values showed mainly low intakes of vitamins A, B9, B12, C, D, E, and of calcium and potassium. The most critical contributions of ricebean and other pulses compared to the recorded levels of inadequate nutrient supplies were vitamin B9, calcium and potassium.

The study contains a number of findings expected to have broader interest, and it will be published as a journal article. A paper on challenges in research, development and promotion of under-utilized crops, using ricebean as a case example was presented at the annual meeting of the American Association of Geographers in Las Vegas in March 2009, and has been invited to peer reviewed publication.

2.5.3 Workpackage progress of the period

The activities in WP5 have primarily been concerned with analysing and reporting data from the dietary surveys. Two deliverables have been submitted during the period, D5.1 and D5.3 which is summarising and concluding the work of WP5.

In addition, Dr. Peter Andersen participated in the annual meeting in Himachal Pradesh in September 2009 as well as the concluding workshop in Kathmandu, January/February 2010.

Deliverable 5.1 was concerned with food preparation and diets. The most common way of consuming ricebean is as a *dal* which is served as a protein rich sauce/soup along with staple grains and vegetables curries, occasionally in addition to animal source foods. It is common practice to soak ricebean before boiling or pan frying, and in some recipes, slight sprouting is performed. Since ricebean contains a considerable amount of phytic acid, these preparations are essential to reduce the anti-nutritive effects that bind up calcium, iron and zinc in complexes that are not bio-available. Also other nutritive parameters are strongly affected by soaking, sprouting and other household level preparations. It has been noted that ricebean in some households may be subject to the trend of pressure cooking without prior soaking/sprouting, which should be discouraged whenever possible.

The question is also relevant with respect to development of new recipes and new semi-processed products made from ricebean, since it is important in order to improve the nutritional quality of these products. It is, however, a question which pertains to all pulses and not to ricebean specifically.

Dehulling and preparation of more processed food items are less common with ricebean, probably because the seed hull is not very easily removed. Demonstration trials carried out in connection with WP5 have shown that popular food items can be made from dehulled and ground ricebean, so there is a need for development of methods for making new, value added products from ricebean in order to reach a larger market and improve the marketing situation.

Deliverable 5.1 also contained some of the general findings concerning the composition of diets in the four areas where the dietary surveys were undertaken: Himachal Pradesh, Assam, Gulmi in the West/Central Hills and Dolakha in the Eastern Hills, Nepal. The diets were found to have similarities in terms of being rice/pulse dominated, but also with some distinct differences. A typology can be outlined as follows:

- Dolakha: *Dal bhat* + maize and wheat, some non-vegetarian
- Gulmi: *Dal bhat* + more maize and wheat, some non-vegetarian
- HP: Rice + wheat bread + *dal* + more pulses, vegetarian
- Assam: Rice, leafy vegetables, fish, pork + pulses but not in every meal

The frequency analysis of pulses consumed showed that lentils are the most important pulse overall, followed by fava bean and cowpea, but there are major regional differences. In Himachal Pradesh, chickpea is very important. Ricebean was the sixth most frequently reported pulse in the interviews, which may reflect that effort have already been made to promote ricebean in the study areas.

Due to their predominance in the diet, the staple grains rice, wheat and maize were in all sites the major source not only of energy (and totalled about 80 % of the calorie intake), but also of protein, single amino acids, and most of the micronutrients. In all the sites, pulses were eaten more or less on a daily basis, but due to the smaller amount, their contribution was nearly as high as that of the staple grains.

Deliverable 5.3 contained the main findings from the dietary survey and compared it with data on ricebean content of different nutrients in order to establish the potential role of ricebean to meet the recorded deficiencies. In addition a comparison was made with other, common pulses, in order to understand what happens if ricebean serves as a substitute rather than an addition to other pulses. In this

respect, it was found that ricebean contains somewhat less protein but with a better content of lysine and other essential amino acids than other pulses studied. It holds a little less of some B vitamin than the average, but not when it comes to folate. The main difference between ricebean and eight other pulses was a substantially higher content of several minerals, especially calcium, magnesium, potassium and iron, while it contains a little less zinc than the eight other pulses investigated. Especially the calcium content is the highest of all pulses.

Regarding the deficiencies, the dietary surveys showed that Protein-Energy Malnutrition (PEM) was less prominent than ‘hidden hunger’ – deficiency of micronutrients. This is in line with contemporary changes in paradigms in nutrition research.

The mean values of intake indicated that the most widespread deficiencies were the

- Vitamins: A, B₉, B₁₂, C, D and E
- Minerals: calcium, iron and potassium.

The recorded intakes of these nutrients were severely below the recommended values for non-pregnant women and even worse compared to the values for pregnant women.

In addition, the intake of fat was low. For other nutrients, the *mean values* were basically close to or above the recommended daily intake for non-pregnant women. However, there were considerable variations in the distribution of intake of the micronutrients, and notably the distribution of intakes of several B vitamins were *skewed downwards so that many women were below the recommendations despite mean values indicating sufficient intakes*.

The *seasonal variations showed no distinctive patterns*, except for vitamin A which in some sites was abundant in the season of for instance sweet potatoes or leafy green vegetables, in other words reflecting a “real” situation.

Comparing the field evidence of deficiencies, we will expect that the most important potential role of ricebean would be with respect to vitamin B’s, especially folate, and the minerals, especially calcium, iron and potassium.

An “impact” study was made on the basis of comparing the calculated daily intake per individual in the dietary survey with what the distribution would be if 30 grams of ricebean were added, based on the assumption that this value could be indicative of a possible increase in pulse intake over the existing. Then, the histograms of calculated intake and calculated improvement of status were compared to the recommended values from the potential contribution from increased ricebean intake was evaluated against the recorded intakes, and the recommended values as taken from National Academy of Sciences (NAS 2004), were added to the histograms in order to relate the intake to bodily requirements:

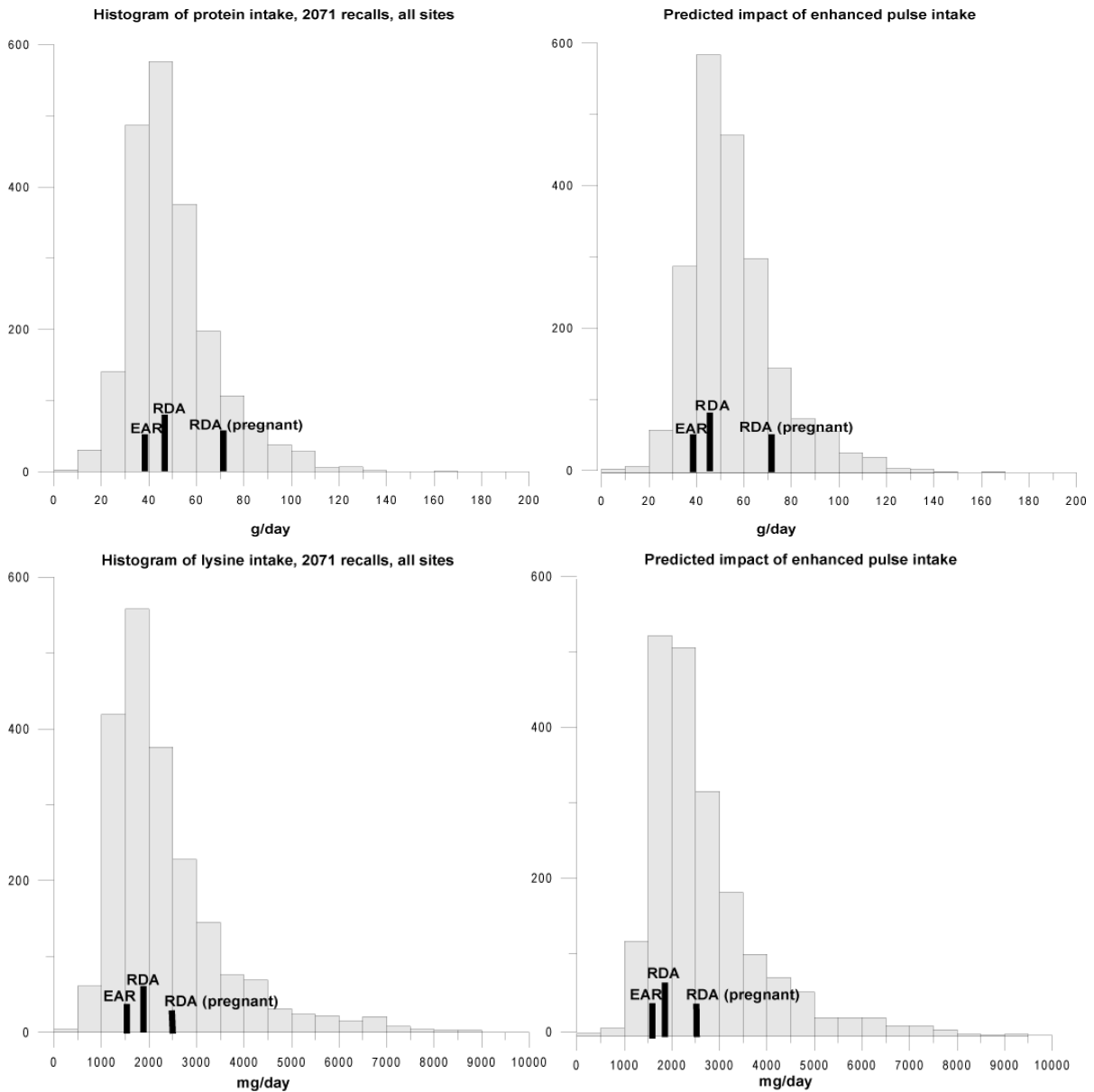
EAR – the Estimated Average Requirement, the value at which about half the women have met their needs of the nutrient.

RDA – the Recommended Dietary Allowance, the level at which almost all (97 or 98 percent) have their needs met

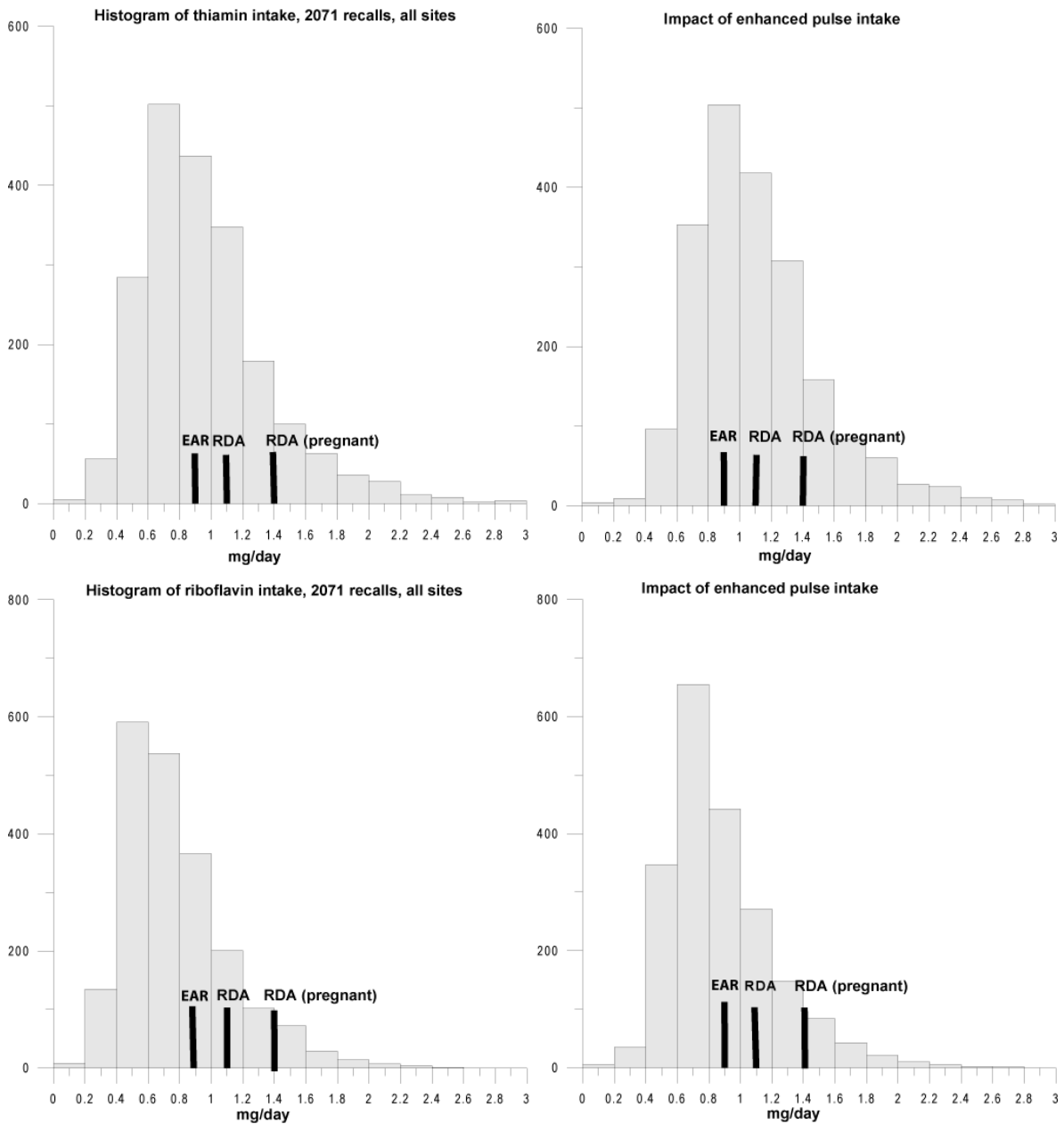
RDA (pregnant) – the RDA for pregnant women

AI – the Adequate Intake, a value believed to cover the needs for all individuals, but lack of data restricts the use of confidence intervals for this group.

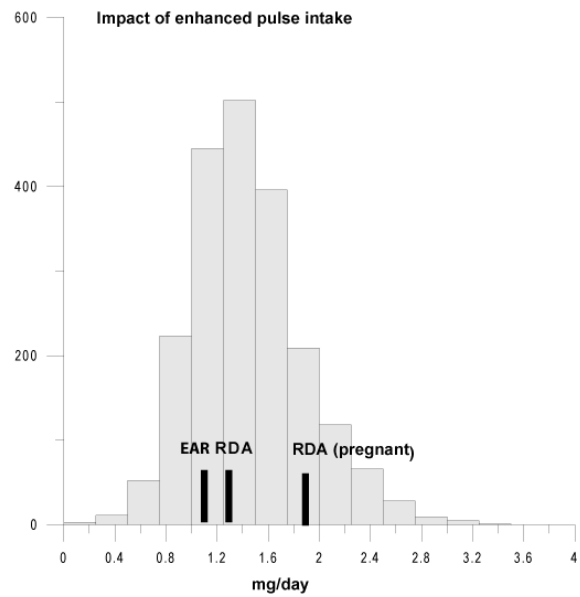
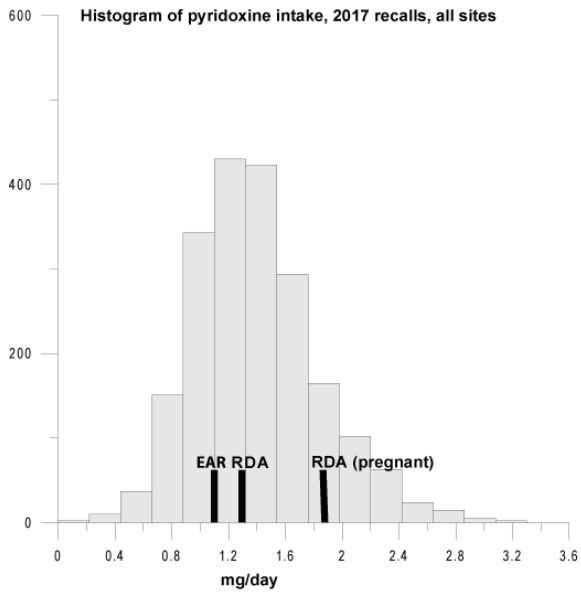
The results for protein and lysine show that the mean intakes were close to sufficiency, but a substantial group of women were consuming somewhat less than the recommended values. When adding the contribution from 30 grams of ricebean (the histogram on the right), a major group were moved from risk status to safe status for non-pregnant women but the effect was less for pregnant women.



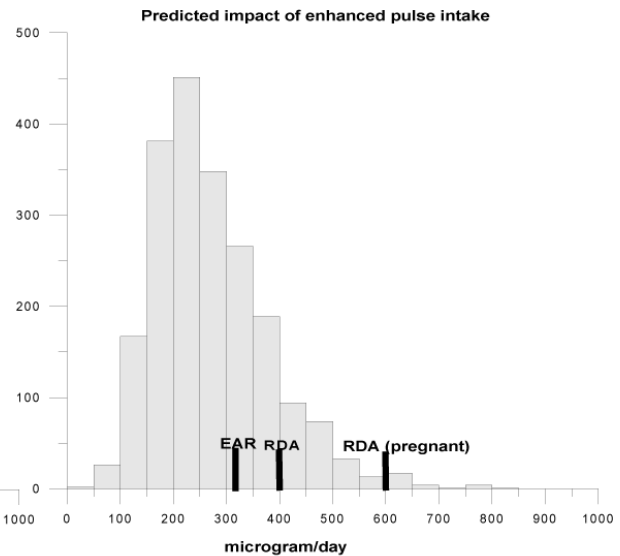
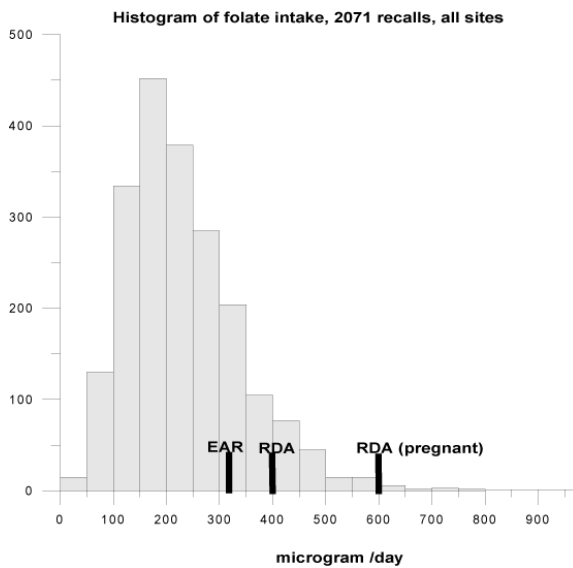
When it comes to vitamin B’s, several of these exhibited a distribution in intakes which was substantially skewed downwards, so even though the mean values indicated only marginal status, a large number of women would fall below the recommended values for non-pregnant women and even more when it comes to pregnant. The diagrams for thiamin (B1) and riboflavin (B2) are examples of this:



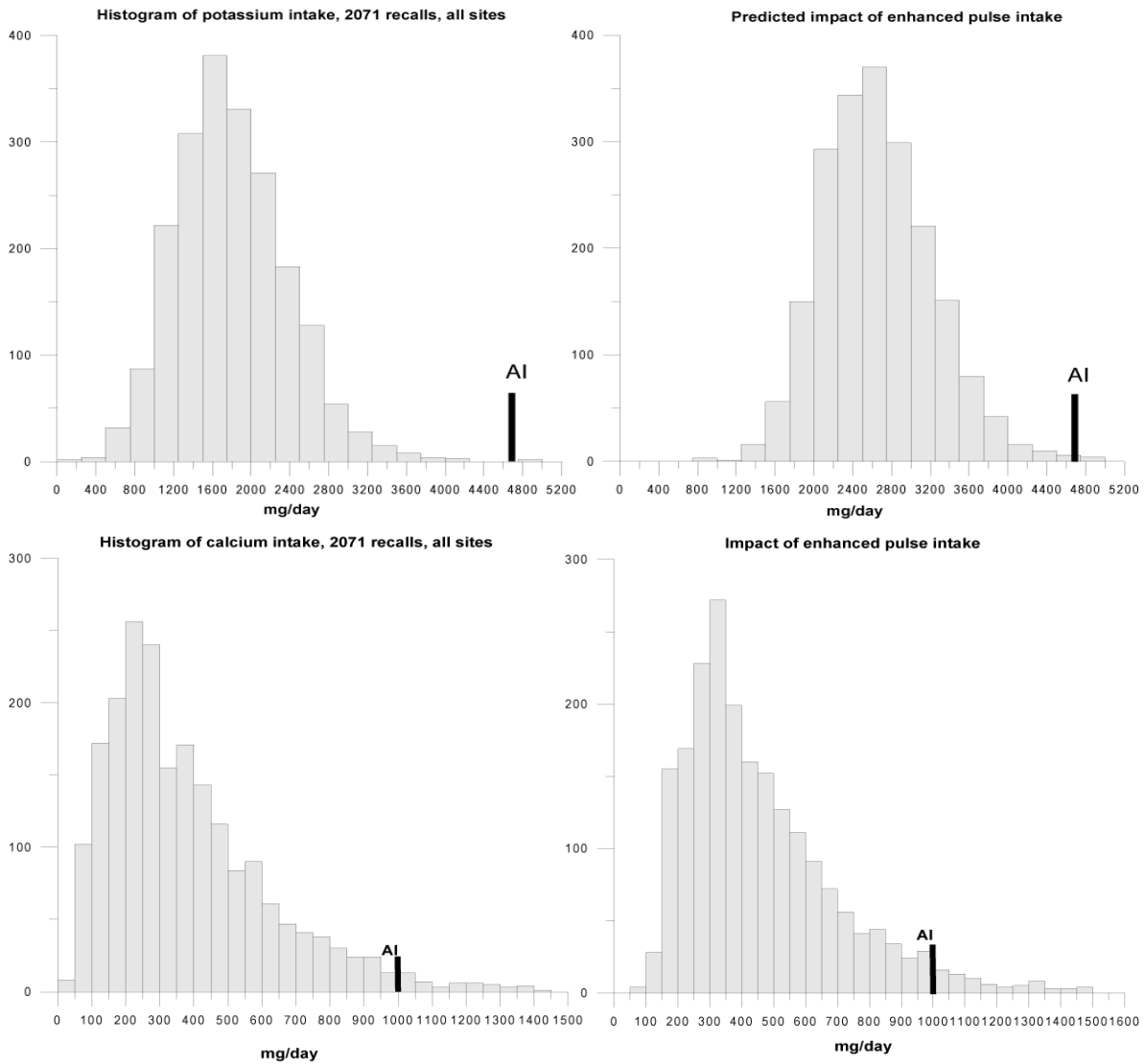
The results for pyridoxine (vitamin B6) shows a distribution curve which is closer to a normal bell-shaped curved, with a mean which is above the RDA value. Adding extra ricebean would move this curve to the right so that a large number of women would move from risk to safe status.



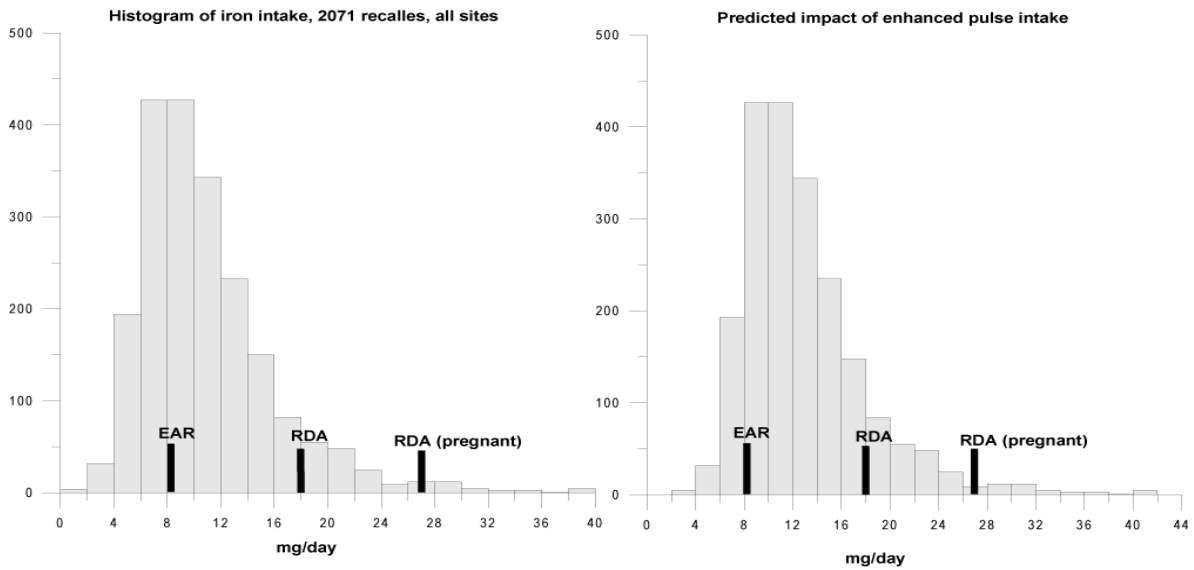
Regarding folate, the predicted level of malnutrition was severe and again with a distribution which was skewed downwards. In this case, despite the good content of folate in ricebean, the calculated effect of adding extra ricebean to the diet would not be enough to move more than a small number above the RDA for non-pregnant, and even less for pregnant women. The lesson from folate is that, although there is a fit between forecasted malnutrition and a good content of ricebean, *a realistic increase in ricebean intake is not enough to cope with severe nutrient deficiencies.*



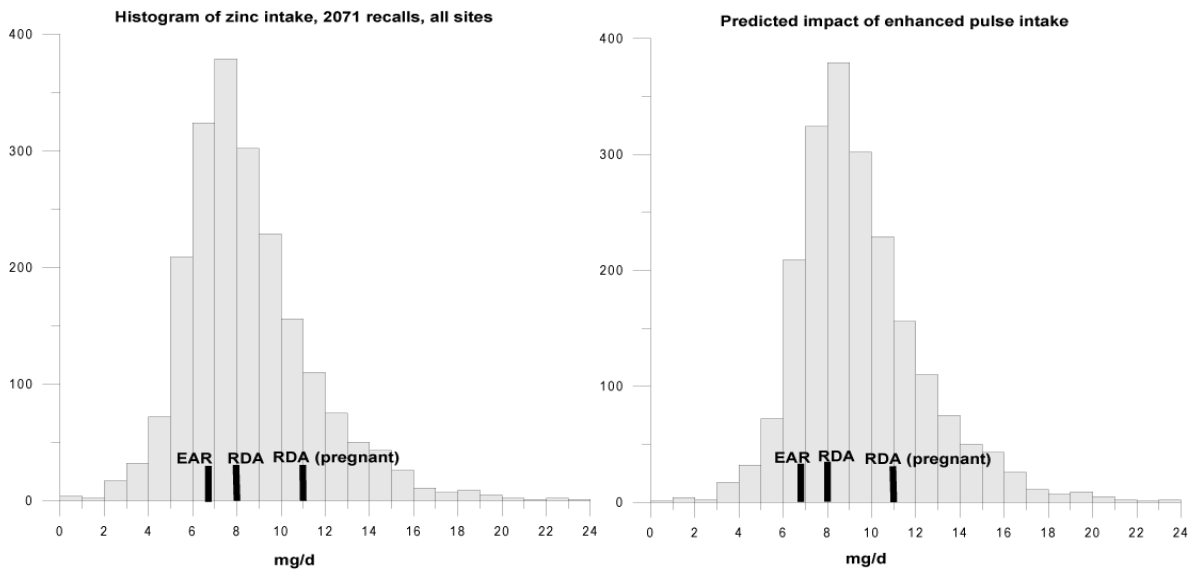
Likewise for potassium and calcium, the level of inadequate intake was severe, and despite a high content of these minerals in ricebean, it could move only a fraction from risk status to above recommended values.



When it comes to iron, there are big differences between values for non-pregnant and pregnant women. The content of ricebean is high relative to other pulses, and adding extra ricebean to the diet could move a number of non-pregnant women from risk to safe status, while still a majority of both non-pregnant and pregnant women would consume below the recommended values.



Finally (of the nutrients presented here), the mean zinc intake was in the adequate range, and additional ricebean intake would potentially move a large number from risk to safe intake levels.



A summary of these calculations can be seen in the following table:

| | % of recalls above RDA/AI | % recalls above RDA/AI with additional 30 g ricebean |
|------------------------------|---------------------------|--|
| Protein ¹ | 50.4 | 66.6 |
| Lysine ¹ | 56.7 | 75.8 |
| Thiamin ¹ | 29.7 | 44.0 |
| Riboflavin ¹ | 15.7 | 21.5 |
| Niacin ¹ | 27.6 | 33.6 |
| Pantotenic acid ² | 57.0 | 61.0 |
| Pyridoxine ¹ | 55.5 | 59.2 |
| Folate ¹ | 8.0 | 11.6 |
| Potassium ² | 0.4 | 0.6 |
| Calcium ² | 2.4 | 3.6 |
| Iron ¹ | 8.6 | 12.6 |
| Zinc ¹ | 49.8 | 68.4 |

% recalls above recommended values. ¹ is RDA for non-pregnant women, ² is AI for non-pregnant women.

This highlights how the *fit* between ricebean and forecast malnutrition problems is a balance between relative dimensions:

- The severity of malnutrition, mean values as well as distribution of intakes
- The differences in needs between non-pregnant and pregnant individuals (and further men and other age groups)
- The nutrient content in ricebean compared to other pulses
- The nutrient content in ricebean in particular and how it can contribute to the nutrient density of the total diet

It is beyond the scope of this workpackage to judge whether some reduction in severe deficiencies (such as calcium and potassium) will have greater health impact than moving a large number of individuals from slightly at risk levels to above recommended values. It can be assumed that this will vary from nutrient to nutrient and even between different health risks associated with the individual nutrients.

One important contribution from the study – in addition to improving the knowledge of the role of pulses in the total diet – may be that the deficiencies encountered can add to the scientific understanding of what nutritional problems that can be expected in these, highly rice dominated diets. While the study confirms that micronutrient deficiencies can be more widespread than Protein-Energy-Malnutrition, some of the severe deficiencies are probably less focused in research and intervention. This relates especially to the highly inadequate intake of for instance calcium, potassium and folate.

Academic dissemination

One paper which was presented in connection with the Association of American Geographers' conference in Las Vegas, March 2009, was invited for publication in a special issue of *International Journal of Agricultural Sustainability*, and the manuscript has been accepted for publication. It will be in print in 2011.

Work from WP5 has also been presented and used in research based teaching, both at Dept. of Geography at University of Bergen, and in interdisciplinary courses at Centre of International Health at University of Bergen.

Post-project work on FOSRIN based activities include presentations of findings at different venues. One presentation of findings will take place at a seminar at the HEMIL Centre (Research Centre for Health Promotion), University of Bergen, June 2010.

An abstract has been submitted to the major conference Tropentag, Zürich, September 2010, which normally is attracting about 1500 researchers. The paper is planned to be elaborated into an article for submission to *Social Science and Medicine* or similar, relevant peer reviewed journal.

2.5.4 Deviations

There were no deviations to the final WP plan for this period.

Table 2.6: Deliverables list for the period

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

*) if available

| Del. no. 19 | Deliverable name | WP no. | Due date ²² (project month) | Actual/Forecast delivery date | Estimated indicative person-months *) | Used indicative person-months *) | Lead participant |
|-------------|--|--------|--|---|---------------------------------------|----------------------------------|------------------|
| D1.1 | Empirically estimated hedonic demand function for legumes | 1 | 27 | Formula. Presented in conference. | 24 | 24 | CAU Kiel |
| D1.2 | Legumes Trait Value Index for guiding ricebean breeding | 1 | 27 | Formula. Presented in conference. | 18 | 16 | CAU Kiel |
| D1.3 | Strategy for introducing ricebean into the legumes supply-chain | 1 | 30 | Integral part of D1.4 | 11 | 8 | CAU Kiel |
| D1.4 | Final report (thesis) on ricebean marketing | 1 | 35 | Currently in final preparation | 15 | 12 | CAU Kiel |
| D1.5 | Article on ricebean marketing in a research journal | 1 | 36 | Cannot follow until D1.4 approved by CAU | 3 | 0 | CAU Kiel |
| D2.1 | National distributions of ricebean published | 2 | 12 | June 2007 | 24 | 24 | LI-BIRD |
| D2.2 | Analysis of local knowledge of rice bean diversity and uses from selected study areas published | 2 | 24 | May 2009 | 45 | 45 | LI-BIRD |
| D2.3 | Analysis of ricebean diversity published in a research journal | 2 | 30 | 40. In preparation | 6 | 1 | LI-BIRD |
| D3.1 | Polymorphic markers identified and published | 3 | 24 | May 2009 | 45 | 45 | NARC |
| D3.2 | Diversity in Nepal and India on the basis of molecular markers described and published | 3 | 36 | Draft submitted with this report | 9 | 7 | NARC |
| D3.3 | Diversity estimates using agromorphological or molecular markers in relation to biophysical and socio-economic variables described and published | 3 | 45 | In preparation. Delayed by power outages and expected July 2010 | 9 | 7 | NARC |

| Del. no. 19 | Deliverable name | WP no. | Due date ²² (project month) | Actual/Forecast delivery date | Estimated indicative person-months *) | Used indicative person-months *) | Lead participant |
|-------------|---|--------|---|---|---------------------------------------|----------------------------------|------------------|
| D4.1 | Quantitative and qualitative data on performance and acceptability of genotypes analysed and published | 4 | 43 | July 2010. Delayed due to power outages in Nepal causing problems with data analysis | | | GVT |
| D4.2 | Quantitative and qualitative data on farmers preferences analysed, compared with results of WP1 and published | 4 | 44 | July 2010. Delayed due to power outages in Nepal causing problems with data analysis | | | GVT |
| D5.1 | Diet and food preparation documented and published | 5 | 24 | November 2009 | 30 | | UB |
| D5.2 | Nutrient content analysed, documented and published | 5 | 30 | May 2009 | 16 | 16 | UB |
| D5.3 | Analysis of health and nutrition impact published | 5 | 29 | Draft submitted with this report | 15 | | UB |
| D7.1 | Dissemination strategy established and reviewed quarterly | 7 | 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 | 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 | 4.5 | 4.5 | CAZS-NR |
| D7.2 | Project website set up | 7 | 3 | 3 and ongoing | 1.5 | 3 | CAZS-NR |
| D7.3 | Project brochures and posters | 7 | 15, 25, 35 | 15 and on-going | 3 | 3 | CAZS-NR |
| D7.4 | Technical documentation for dissemination to farmers and extension workers | 7 | 15, 25, 35 | 15 and on-going | 3 | 4 | CAZS-NR |
| D7.5 | Press articles and broadcasts | 7 | 6, 18, 30 | 18 and ongoing | 2 | 2 | CAZS-NR |
| D7.6 | Demonstrations “on-farm” | 7 | 6, 18, 30 | 18 and ongoing | 5 | 5 | CAZS-NR |
| D7.7 | Outputs on CD-Rom and/or video | 7 | 18 | September 2009 | 2 | 2 | CAZS-NR |
| D7.8 | Final workshop proceedings | 7 | 48 | In preparation. To be submitted for publication late 2010 | 2 | 2 | CAZS-NR |

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 | 40 | UB |
| 10 | Identification of polymorphic markers | WP3 | 12 | 24 | NARC |
| 11 | Annual meeting | WP6 | 12 | 18, 30, 42 | CAZS-NR / all |
| 12 | Completion of mother and baby trials | WP4 | 24, 36, 48 | 24, 36, 48 | GVT |
| 13 | Membership of networks | WP6 | 12 | Ongoing | All |
| 14 | 2nd diet and nutrition report | WP5 | 24 | 36 | UB |
| 15 | Lab analyses of nutrient contents | WP5 | 17 | 17 | UB |
| 16 | Complete supply-chain field work | WP1 | 18 | 42 | CAU |
| 17 | Complete field work for agro-morphological characterisation | WP2 | 18, 24 | 18, 24, 36, 48 | LI-BIRD |
| 18 | Germplasm evaluation with markers | WP3 | 24 | 42 | NARC |
| 19 | Field trials for effect of sowing date completed | WP2 | 46 | 46 | LI-BIRD |
| 20 | Studies of outcrossing and population genetics completed | WP4 | 46 | 46 | NARC |
| 21 | Study of hardseededness completed | WP4 | 46 | 46 | GVT |

| | | | | | |
|----|---|-------|----|----|---------|
| 23 | Evaluation of stratified germplasm sample with identified markers | WP3 | 45 | 45 | NARC |
| 24 | MLTVI quantified | WP1 | 27 | 45 | CAU |
| 25 | Molecular marker data analysed | WP3 | 45 | 45 | NARC |
| 26 | Strategy for ricebean introduction designed and reviewed | WP1 | 42 | 48 | CAU |
| 27 | Analysis of data from mother and baby trials and comparison with results of WP2 | WP2/4 | 46 | | CAZS-NR |
| 28 | Synthesis of data: potential impacts | WP5 | 34 | 48 | UB |
| 29 | Thesis submitted | WP1 | 45 | 50 | CAU |
| 30 | Final workshop held | WP6 | 47 | 47 | CAZS-NR |

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 4.

To hold the 4th Annual Meeting. This was held at the Hotel Anand Palace, Bhagsu nag Dharamshala, Himachal Pradesh, India from September 13 – 15, 2009. Teams from all partners attended: unfortunately Prof Yadavendra was unable to travel due to illness, Dr Joshi was detained in quarantine in Delhi due to suspected H1N1, and Prof Mueller was unable to travel due to an airline pilots strike. Despite these absences, a useful and constructive meeting was held with useful discussions and interaction between participants. The meeting included a field tour to ricebean growing areas and to the University Research farm in Palampur.

To hold the Final Workshop. This was held from 31 January to 4 February 2010, at the Hotel Country Villa, Nagarkot, Bhaktapur, Nepal. As well as project staff, two senior scientists from India, Dr M Dutta, Technical Programme Leader, All India Coordinated Research Programme on Underutilized Crops in GB Pantnagar University of Agriculture, Rani-Chauri, Uttarakhand, of the All-India and Dr RP Dua, Project Coordinator, All India Coordinated Network on Underutilized Crops, NBPGR New Delhi attended and gave presentations, as did Dr Bhuwan Sthapit from Bioversity International and several senior staff from NARC in Nepal. All presentations are available on the project website at <http://www.ricebean.org/publication.htm#presentations>.

To elaborate the detailed workplan. This was adjusted again at the 4th Annual Meeting, and the updated barchart is attached. Experimental protocols were reviewed and updated if necessary at Technical Meetings.

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 participate in the Asia Pacific Mountain Forum (<http://www.mtnforum.org/rn/apmn.cfm>) from ICIMOD. Other networks include the International Centre for Underutilised 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). Project deliverables previously submitted to the website of the GFU are awaiting the new site for ICUC. Dr Joshi in Kathmandu is actively building stronger linkages with ICIMOD, and project deliverables have been published on their website at

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, 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.

A major additional activity was the development of a follow-on project for submission under the 2009-2010 Global Programme on Agricultural Research for Development (ARD), which was submitted in January. This involved the current project partners, plus additional partners from Thailand.

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 years 2, 3 and 4. The Asian partners are particularly experienced 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 have been 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 (May 2010) received over 16,000 hits (verified by <http://www.statcounter.com>). Content includes staff and institutional profiles, a description of the project, including a Google Maps link to field sites, 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 either to abstracts or to full papers where copyright restrictions permit. It also includes a page on hill farming in India and Nepal, a page on the nutritional aspects, and a link to the ricebean Wikipedia entry was added. Work to provide a secure area for to allow project staff to exchange documents and other material that needs to remain confidential was shelved due to the ready availability of other means of communication, and administrative delays with IT support for this in Bangor.

Deliverable 7.7 (Outputs on CD-ROM / video) has been completed (see below) and was uploaded to the project website in September 2009.

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 India, and in Nepal.

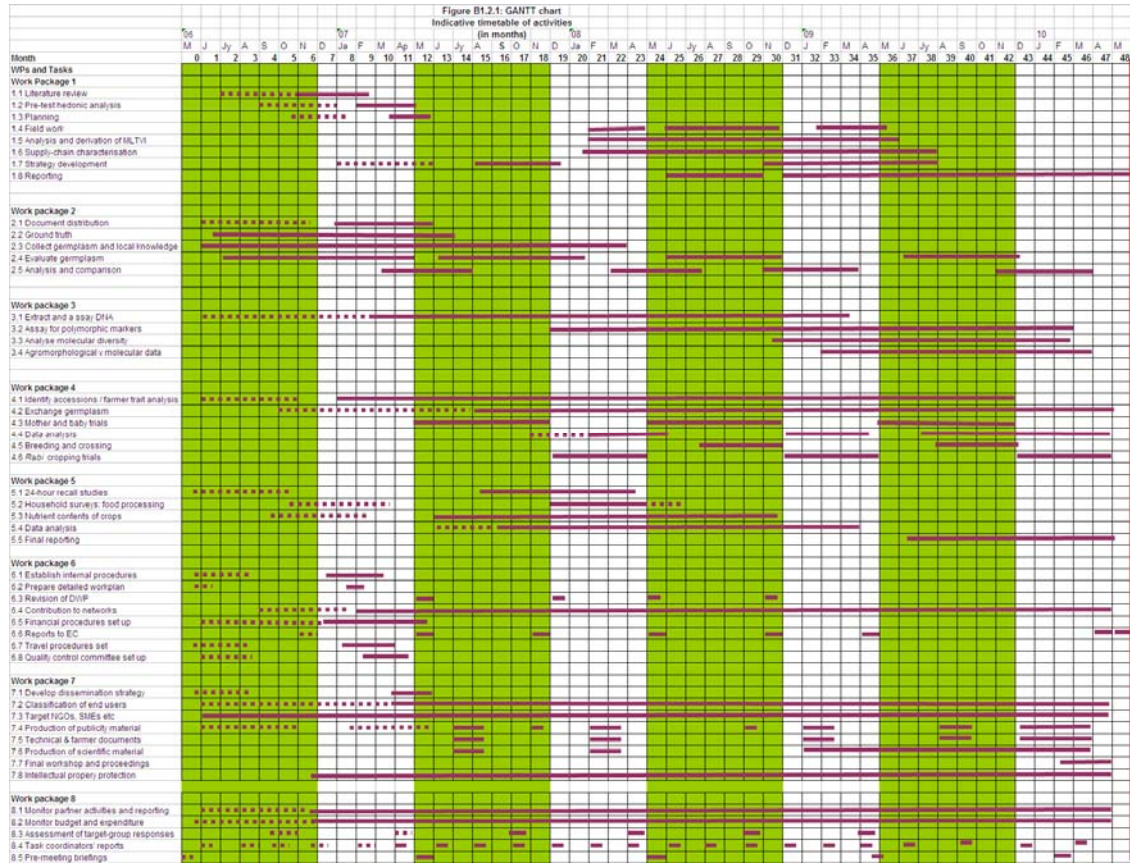
3.2 Contractors: Comments regarding contributions, changes in responsibilities and changes to consortium itself, if any

There have been no changes in either contractors' responsibilities or to the consortium itself. After the delays in year 1 in India in particular, things progressed well. There have been no significant problems with any of the contractors failing to meet obligations.

3.3 Project timetable and status, including an updated, frontlined barchart. Clarify changes and impact on the planned milestones, if any

Figure 3.1 shows the updated final barchart for comparison with that submitted in the previous years (Figures 3.2, 3.3 and 3.4)

Figure 3.1 Workpackages - Plan and Status Barchart at end of project



In the chart, the green colour represents the growing season for the crop

Figure 3.2: Workpackages - Plan and Status Barchart after year 3

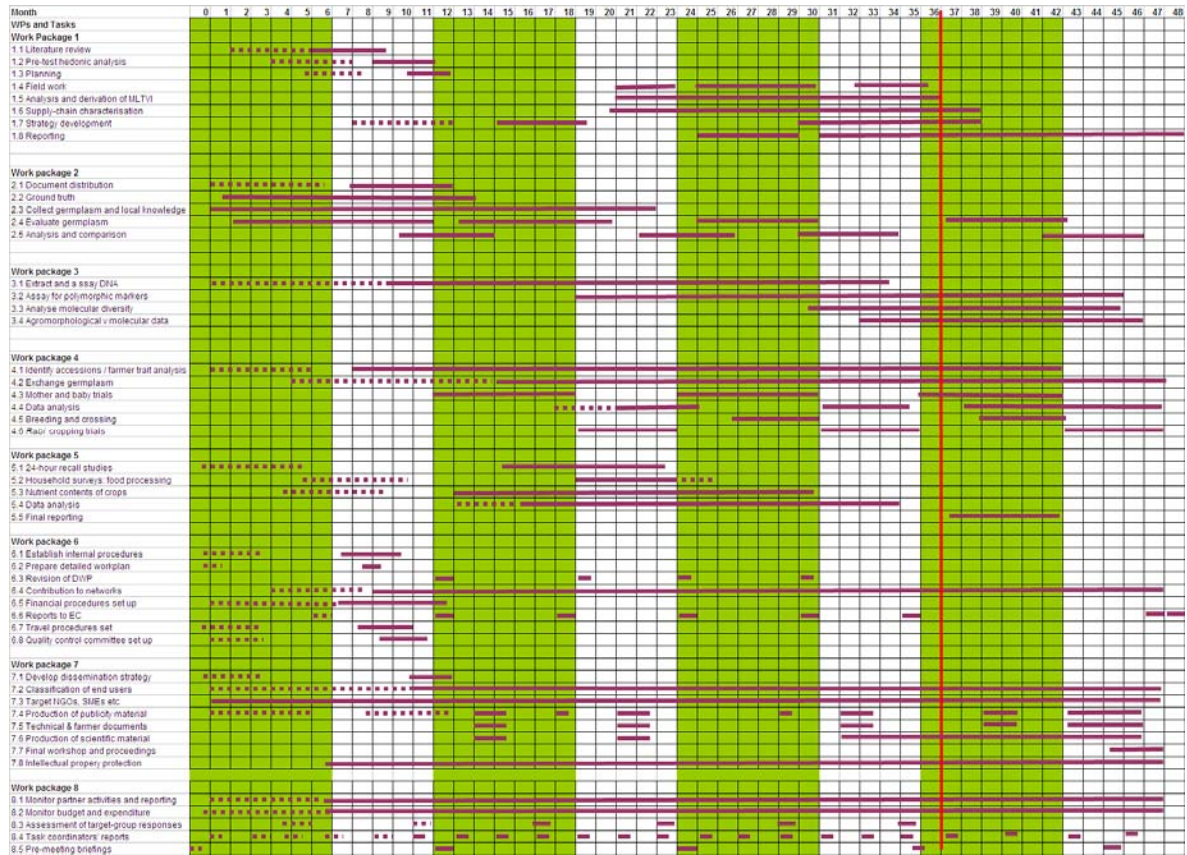
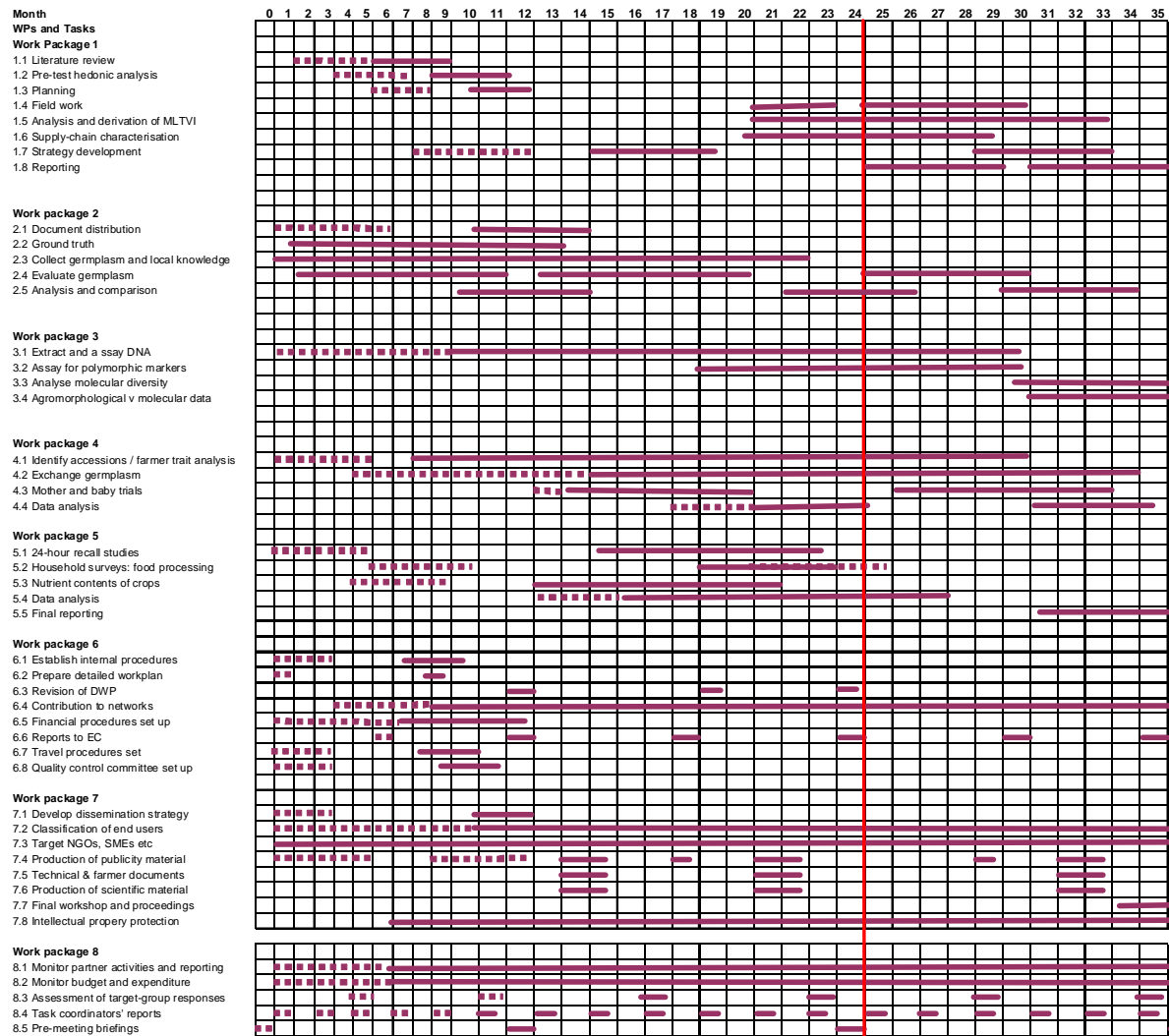


Figure 3.3: Workpackages - Plan and Status Barchart after year 2



India. Our intention is to publish the proceedings either as a special issue of a scientific journal, or as individual papers. In this way we will achieve more impact than through publishing in book form.

Table 3.4.1. Details of meetings held in year 4

| Date | Location | Attendance | Purpose | Management outcomes* |
|-------------------------|---|---|--|---|
| April 8, 2009 | Anand | Dr JP Yadavendra (GVT); Dr N Kumar (CSKHPKV); Dr SB Neog (AAU) | Annual technical programme meeting of the Indian partners | <i>Scientific only</i> |
| September 13 – 15, 2009 | Hotel Anand Palace, Bhagsu nag Dharamshala, Himachal Pradesh, India | Dr PA Hollington (Coordinator), Prof JR Witcombe, (CAZS NR); Ms D Bürgelt, Ms Ritu Gulati (CAU); Dr P Andersen (UB); Dr S B Neog, (AAU Jorhat); Mr Amar Prasad (GVT Dahod); Dr N Kumar, (CSJHPKV, Palampur); Mr K Khadkha (LI-BIRD, Pokhara); Dr J Bajracharya (NARC Kathmandu) | 4 th annual meeting. Administrative and budget reports for year 3; Overview of scientific progress, Status of deliverables, Detailed scientific reports by WP, Dissemination and outreach activities, Plan for disseminating knowledge; Plans for next reporting period; Plans for final workshop; Plans for follow-up project; Issues directly affecting farmers and industry; field visit to ricebean growing areas | Partners agreed to contribute additional website pages. Review timings of deliverables and workplan adjustments Format of new proposal agreed Final workshop agreed |
| 25 – 26 May, 2009 | Himachal Pradesh, India | Dr JP Yadavendra, Dr N Kumar, Dr SB Neog | Monitoring and evaluation of field sites | <i>Scientific only</i> |
| Feb 1 – 3, 2010 | Nagarkot, Kathmandu, Nepal | Dr PA Hollington (Coordinator), Prof JR Witcombe, Dr KD Joshi (CAZS NR); Mr K Khadkha (LI-BIRD, Pokhara); Dr J Bajracharya (NARC Kathmandu) + others | Final workshop: full progress reports on project plus presentations on WP outcomes | Scientific, plus discussion of new equipment for NARC |

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

Section 4 – Other issues

Not relevant to this report

Annex 1. Mean performance of germplasm lines and checks of ricebean at Palampur, HP (2009)

| Entries | 50% Flowering | Flowering Period | Days to first Mature pod | Terminal Leaflet Blade Length (cm) | Terminal Leaflet Blade Width (cm) | Plant Height (cm) | Pods/ plant | Pods Length (cm) | 100-seed Weight (g) | Grain yield (t/ha) |
|------------|---------------|------------------|--------------------------|------------------------------------|-----------------------------------|-------------------|-------------|------------------|---------------------|--------------------|
| RBHP-1 | 82 | 37 | 105 | 8.1 | 5.8 | 70 | 30 | 7.7 | 4.6 | 0.71 |
| RBHP-2 | 79 | 48 | 104 | 9.7 | 6.5 | 117 | 126 | 8.4 | 6.0 | 1.24 |
| RBHP-3 | 77 | 49 | 106 | 8.9 | 6.4 | 147 | 70 | 8.3 | 5.2 | 1.53 |
| RBHP-5 | 86 | 31 | 106 | 7.8 | 6.0 | 66 | 19 | 8.3 | 4.7 | 0.50 |
| RBHP-6 | 82 | 47 | 95 | 8.8 | 5.6 | 70 | 37 | 8.0 | 4.8 | 0.62 |
| RBHP-7 | 81 | 47 | 105 | 9.6 | 6.7 | 142 | 126 | 8.6 | 5.0 | 2.15 |
| RBHP-8 | 78 | 56 | 98 | 8.9 | 6.3 | 104 | 51 | 7.9 | 5.5 | 0.75 |
| RBHP-9 | 80 | 37 | 104 | 8.1 | 5.5 | 98 | 53 | 7.3 | 4.0 | 1.02 |
| RBHP-10 | 81 | 33 | 99 | 7.6 | 5.5 | 107 | 28 | 7.0 | 7.3 | 0.91 |
| RBHP-11 | 82 | 37 | 104 | 8.5 | 5.9 | 101 | 58 | 7.5 | 4.4 | 1.01 |
| RBHP-12 | 80 | 33 | 105 | 8.4 | 6.5 | 112 | 81 | 8.5 | 6.3 | 0.80 |
| RBHP-13 | 81 | 48 | 98 | 9.2 | 6.5 | 102 | 22 | 6.7 | 4.8 | 0.81 |
| RBHP-14 | 82 | 27 | 108 | 6.7 | 5.0 | 76 | 22 | 7.6 | 4.2 | 0.46 |
| RBHP-16 | 86 | 47 | 105 | 8.4 | 6.1 | 69 | 40 | 8.6 | 5.4 | 0.77 |
| RBHP-17 | 82 | 35 | 109 | 8.5 | 6.0 | 75 | 28 | 8.3 | 4.7 | 0.81 |
| RBHP-18 | 82 | 47 | 95 | 7.5 | 5.2 | 63 | 21 | 7.8 | 4.5 | 0.89 |
| RBHP-19 | 86 | 39 | 104 | 7.7 | 5.4 | 63 | 30 | 8.0 | 5.4 | 0.66 |
| RBHP-20 | 85 | 30 | 104 | 8.0 | 5.2 | 60 | 28 | 8.3 | 5.2 | 1.02 |
| RBHP-22 | 81 | 37 | 104 | 8.4 | 6.0 | 87 | 35 | 7.9 | 5.0 | 0.67 |
| RBHP-23 | 84 | 27 | 106 | 8.6 | 5.7 | 51 | 27 | 8.4 | 5.9 | 0.70 |
| RBHP-24 | 81 | 34 | 107 | 7.7 | 5.5 | 65 | 39 | 7.0 | 4.4 | 0.68 |
| RBHP-25 | 84 | 33 | 105 | 8.3 | 6.1 | 58 | 30 | 6.9 | 4.2 | 0.60 |
| RBHP-26 | 80 | 47 | 95 | 8.1 | 5.6 | 69 | 27 | 8.1 | 4.4 | 0.58 |
| RBHP-27 | 82 | 34 | 105 | 9.3 | 6.0 | 107 | 82 | 7.9 | 5.7 | 0.89 |
| RBHP-29 | 85 | 36 | 105 | 8.3 | 6.0 | 68 | 38 | 7.9 | 5.8 | 1.25 |
| RBHP-30 | 79 | 37 | 97 | 9.0 | 6.2 | 69 | 32 | 8.3 | 4.9 | 0.97 |
| RBHP-31 | 85 | 45 | 98 | 8.7 | 5.8 | 96 | 46 | 8.2 | 5.9 | 0.72 |
| RBHP-32 | 86 | 45 | 105 | 8.2 | 5.8 | 65 | 20 | 7.3 | 5.4 | 0.54 |
| RBHP-35 | 86 | 48 | 98 | 8.9 | 6.6 | 85 | 45 | 7.7 | 5.2 | 0.53 |
| RBHP-36 | 81 | 46 | 97 | 8.0 | 5.3 | 88 | 37 | 8.6 | 6.7 | 0.84 |
| RBHP-37 | 86 | 32 | 104 | 9.2 | 6.2 | 80 | 27 | 9.3 | 5.8 | 0.63 |
| RBHP-38 | 85 | 54 | 105 | 9.0 | 5.7 | 93 | 29 | 9.2 | 5.8 | 1.22 |
| RBHP-39 | 81 | 34 | 106 | 8.2 | 5.9 | 89 | 55 | 7.6 | 4.0 | 0.90 |
| RBHP-41 | 82 | 49 | 96 | 8.1 | 6.2 | 75 | 36 | 10.1 | 6.4 | 1.08 |
| RBHP-42 | 84 | 34 | 99 | 8.5 | 5.4 | 85 | 38 | 7.1 | 5.5 | 1.06 |
| RBHP-43 | 81 | 50 | 96 | 8.0 | 4.6 | 106 | 28 | 8.9 | 5.8 | 1.32 |
| RBHP-44-A | 84 | 40 | 106 | 8.4 | 6.1 | 96 | 19 | 9.0 | 5.8 | 1.08 |
| RBHP-44(B) | 77 | 50 | 96 | 8.2 | 6.4 | 120 | 78 | 10.2 | 6.5 | 0.94 |
| RBHP-44C | 82 | 45 | 97 | 7.3 | 5.3 | 86 | 23 | 9.7 | 5.9 | 0.58 |
| RBHP – 45 | 81 | 54 | 96 | 7.9 | 5.2 | 73 | 35 | 9.8 | 7.6 | 0.54 |
| RBHP-46 | 85 | 37 | 101 | 8.4 | 5.7 | 65 | 18 | 8.5 | 5.8 | 0.66 |
| RBHP-47 | 82 | 43 | 98 | 7.7 | 5.7 | 85 | 18 | 7.7 | 6.5 | 0.53 |
| RBHP-48 | 85 | 48 | 99 | 8.5 | 4.6 | 91 | 40 | 9.5 | 5.9 | 0.74 |
| RBHP-49 | 82 | 29 | 107 | 7.9 | 5.6 | 71 | 29 | 7.4 | 4.0 | 0.55 |

| Entries | 50% Flowering | Flowering Period | Days to first Mature pod | Terminal Leaflet Blade Length (cm) | Terminal Leaflet Blade Width (cm) | Plant Height (cm) | Pods/ plant | Pods Length (cm) | 100-seed Weight (g) | Grain yield (t/ha) |
|------------|---------------|------------------|--------------------------|------------------------------------|-----------------------------------|-------------------|-------------|------------------|---------------------|--------------------|
| RBHP-50 | 84 | 44 | 102 | 9.1 | 6.4 | 98 | 35 | 9.8 | 6.7 | 0.78 |
| RBHP-51 | 85 | 32 | 103 | 9.2 | 6.2 | 127 | 50 | 9.7 | 5.6 | 1.14 |
| RBHP-52 | 82 | 44 | 105 | 7.9 | 6.2 | 96 | 35 | 7.2 | 6.1 | 0.81 |
| RBHP-53 | 82 | 46 | 98 | 8.0 | 5.9 | 86 | 74 | 8.6 | 6.0 | 1.08 |
| RBHP-54 | 82 | 51 | 97 | 8.7 | 6.3 | 132 | 57 | 9.5 | 5.8 | 0.88 |
| RBHP-55 | 85 | 43 | 97 | 7.9 | 5.8 | 121 | 35 | 8.8 | 6.7 | 0.76 |
| RBHP-56 | 85 | 48 | 97 | 8.7 | 6.2 | 97 | 46 | 8.1 | 6.9 | 0.56 |
| RBHP-58 | 85 | 49 | 99 | 8.1 | 5.7 | 111 | 41 | 8.5 | 5.5 | 0.63 |
| RBHP-59 | 81 | 42 | 101 | 8.2 | 5.3 | 85 | 29 | 7.9 | 4.6 | 0.81 |
| RBHP-60 | 86 | 45 | 98 | 8.3 | 6.0 | 83 | 40 | 9.2 | 5.3 | 0.79 |
| RBHP-61 | 82 | 42 | 99 | 9.5 | 6.7 | 106 | 31 | 8.7 | 6.5 | 0.64 |
| RBHP-62(A) | 81 | 51 | 96 | 8.7 | 6.1 | 94 | 35 | 9.0 | 5.4 | 0.64 |
| RBHP-62(B) | 92 | 29 | 114 | 7.2 | 5.8 | 51 | 22 | 8.2 | 5.5 | 0.34 |
| RBHP-63 | 81 | 48 | 95 | 9.3 | 6.7 | 105 | 32 | 9.5 | 6.3 | 0.97 |
| RBHP-64 | 86 | 36 | 105 | 9.1 | 6.4 | 134 | 43 | 9.1 | 6.5 | 0.96 |
| RBHP-65 | 81 | 47 | 100 | 8.6 | 5.8 | 97 | 38 | 7.1 | 4.7 | 0.57 |
| RBHP-66 | 81 | 33 | 99 | 9.9 | 6.3 | 58 | 43 | 8.7 | 5.8 | 1.14 |
| RBHP-67 | 85 | 32 | 113 | 8.8 | 5.7 | 83 | 69 | 8.7 | 6.1 | 0.98 |
| RBHP-69 | 82 | 39 | 99 | 9.1 | 6.0 | 81 | 35 | 8.8 | 5.0 | 0.68 |
| RBHP-70 | 86 | 33 | 110 | 6.7 | 5.3 | 54 | 30 | 8.2 | 4.8 | 0.74 |
| RBHP-71 | 85 | 32 | 105 | 9.4 | 6.8 | 90 | 51 | 8.4 | 6.1 | 0.67 |
| RBHP-72 | 80 | 42 | 97 | 9.9 | 6.5 | 83 | 26 | 9.7 | 6.0 | 1.23 |
| RBHP-73 | 86 | 46 | 97 | 8.5 | 5.3 | 83 | 29 | 9.1 | 6.6 | 0.56 |
| RBHP-74 | 81 | 37 | 106 | 8.6 | 5.8 | 58 | 24 | 7.9 | 4.6 | 0.78 |
| RBHP-75 | 72 | 30 | 105 | 8.3 | 5.6 | 70 | 33 | 8.3 | 3.6 | 0.81 |
| RBHP-76 | 85 | 32 | 111 | 7.9 | 5.5 | 62 | 27 | 7.1 | 5.5 | 1.26 |
| RBHP-77 | 82 | 31 | 105 | 8.3 | 5.8 | 69 | 28 | 6.5 | 5.2 | 0.65 |
| RBHP-78 | 79 | 34 | 104 | 8.4 | 5.6 | 110 | 64 | 7.7 | 6.1 | 0.84 |
| RBHP-79 | 82 | 28 | 98 | 8.6 | 5.4 | 89 | 21 | 7.7 | 5.2 | 0.76 |
| RBHP-80 | 80 | 46 | 98 | 8.0 | 5.4 | 83 | 26 | 8.9 | 6.1 | 0.60 |
| RBHP-81 | 86 | 25 | 115 | 8.9 | 6.0 | 75 | 48 | 6.8 | 5.1 | 0.44 |
| RBHP-82 | 81 | 31 | 110 | 8.7 | 5.9 | 98 | 66 | 8.4 | 5.2 | 0.74 |
| RBHP-83 | 86 | 31 | 112 | 9.0 | 5.7 | 68 | 38 | 8.4 | 4.8 | 0.39 |
| RBHP-84 | 84 | 37 | 97 | 8.9 | 6.0 | 72 | 40 | 7.8 | 5.1 | 0.59 |
| RBHP-85 | 86 | 30 | 114 | 9.3 | 6.5 | 92 | 38 | 8.2 | 6.4 | 0.97 |
| RBHP-86 | 79 | 40 | 107 | 9.2 | 5.8 | 138 | 106 | 8.3 | 5.6 | 1.81 |
| RBHP-87 | 86 | 31 | 111 | 9.3 | 6.7 | 82 | 99 | 9.2 | 5.4 | 0.94 |
| RBHP-88 | 88 | 31 | 112 | 9.3 | 6.3 | 93 | 38 | 7.6 | 5.5 | 0.54 |
| RBHP-89 | 80 | 41 | 98 | 9.2 | 5.0 | 71 | 16 | 8.1 | 4.8 | 0.72 |
| RBHP-90 | 87 | 32 | 114 | 8.0 | 4.3 | 79 | 30 | 7.7 | 6.0 | 0.52 |
| RBHP-93 | 89 | 40 | 100 | 9.0 | 5.5 | 77 | 32 | 7.4 | 4.7 | 0.80 |
| RBHP-94 | 82 | 28 | 108 | 8.8 | 5.6 | 74 | 47 | 8.5 | 4.9 | 0.63 |
| RBHP-95 | 81 | 30 | 104 | 8.0 | 5.7 | 103 | 15 | 8.3 | 5.2 | 0.74 |
| RBHP-96 | 82 | 31 | 103 | 8.5 | 5.3 | 65 | 29 | 6.9 | 3.1 | 0.42 |
| RBHP-97 | 82 | 48 | 96 | 9.9 | 6.5 | 84 | 39 | 9.8 | 5.9 | 0.75 |

| Entries | 50% Flowering | Flowering Period | Days to first Mature pod | Terminal Leaflet Blade Length (cm) | Terminal Leaflet Blade Width (cm) | Plant Height (cm) | Pods/ plant | Pods Length (cm) | 100-seed Weight (g) | Grain yield (t/ha) |
|----------|---------------|------------------|--------------------------|------------------------------------|-----------------------------------|-------------------|-------------|------------------|---------------------|--------------------|
| RBHP-99 | 80 | 34 | 105 | 11.0 | 5.2 | 77 | 62 | 8.3 | 6.0 | 0.85 |
| RBHP-100 | 80 | 45 | 95 | 7.8 | 5.3 | 72 | 21 | 8.4 | 6.2 | 0.61 |
| RBHP-101 | 87 | 31 | 105 | 9.2 | 6.4 | 107 | 39 | 8.8 | 8.2 | 0.39 |
| RBHP-102 | 87 | 31 | 113 | 8.8 | 6.5 | 67 | 27 | 9.3 | 6.6 | 0.66 |
| RBHP-103 | 92 | 28 | 116 | 8.3 | 5.8 | 92 | 23 | 7.8 | 4.9 | 0.64 |
| RBHP-104 | 91 | 32 | 112 | 7.8 | 5.3 | 81 | 30 | 7.9 | 5.4 | 0.55 |
| RBHP-105 | 91 | 40 | 101 | 8.5 | 5.7 | 81 | 34 | 7.8 | 7.7 | 0.32 |
| RBHP-106 | 82 | 39 | 104 | 8.0 | 5.6 | 45 | 27 | 8.2 | 5.5 | 0.64 |
| RBHP-107 | 85 | 30 | 110 | 8.7 | 5.8 | 65 | 25 | 8.3 | 5.0 | 0.53 |
| RBHP-108 | 83 | 30 | 107 | 8.4 | 5.5 | 75 | 32 | 8.2 | 5.0 | 0.54 |
| RBHP-109 | 87 | 28 | 118 | 8.8 | 5.6 | 97 | 72 | 8.2 | 5.3 | 0.65 |
| RBHP-110 | 81 | 29 | 113 | 8.4 | 5.6 | 102 | 40 | 8.2 | 5.1 | 1.03 |
| RBHP-111 | 82 | 38 | 107 | 8.5 | 5.5 | 118 | 43 | 8.5 | 8.0 | 0.52 |
| RBHP-112 | 80 | 35 | 106 | 9.0 | 8.7 | 79 | 44 | 8.5 | 4.6 | 1.45 |
| RBHP-113 | 82 | 42 | 106 | 8.6 | 6.1 | 88 | 57 | 6.6 | 6.3 | 0.93 |
| RBHP-114 | 82 | 33 | 106 | 8.9 | 5.8 | 73 | 52 | 7.8 | 6.6 | 0.90 |
| BRS I | 84 | 38 | 103 | 8.9 | 6.5 | 102 | 50 | 9.0 | 6.0 | 1.05 |
| BRS II | 80 | 50 | 96 | 8.5 | 6.0 | 89 | 41 | 9.1 | 6.3 | 0.71 |
| Nainy | 83 | 48 | 97 | 8.6 | 5.9 | 101 | 40 | 8.7 | 5.8 | 0.68 |

Mean performance of germplasm lines at village Bhagor, Jhabua, MP.

| Name | Days to flowering | Height (cm) | Type of maturity | Growth habit | Maturity | Seed Colour | Seed size | Seed yield /plant (g) |
|-------|-------------------|-------------|------------------|--------------|----------|--------------|-----------|-----------------------|
| JR-1 | 55 | 93 | C | SE | 95 | Light yellow | Medium | 80 |
| JR-2 | 63 | 119 | C | E | 100 | Brown | Medium | 108 |
| JR-3 | 61 | 93 | C | SE | 95 | Yellow | Small | 80 |
| JR-4 | 61 | 102 | C | E | 124 | Grey | Medium | 75 |
| JR-5 | 59 | 82 | C | E | 135 | Brown | Small | 75 |
| JR-6 | 73 | 97 | C | SE | 145 | Yellow | Medium | 120 |
| JR-7 | 68 | 93 | C | SE | 140 | Brown | Small | 90 |
| JR-8 | 66 | 118 | C | SE | 140 | Light brown | Medium | 80 |
| JR-9 | 58 | 92 | C | E | 130 | Yellow | Small | 70 |
| JR-10 | 61 | 104 | C | SE | 97 | Brown | Small | 75 |
| JR11 | 82 | 76 | C | E | 140 | Variegated | Small | 100 |
| JR-12 | 66 | 106 | C | SE | 100 | Blackish | Small | 120 |
| JR-13 | 62 | 98 | C | SE | 98 | Brown | Small | 80 |
| JR14 | 57 | 103 | C | E | 96 | Yellow | Small | 75 |
| JR15 | 64 | 99 | C | E | 89 | Yellow | medium | 80 |
| JR-16 | 62 | 101 | C | E | 90 | Yellow | Small | 75 |
| JR-20 | 60 | 95 | C | E | 135 | Black brown | Medium | 80 |
| JR-25 | 71 | 78 | C | E | 95 | LightYellow | Small | 100 |
| JR-26 | 62 | 74 | C | E | 80 | Black brown | Small | 85 |
| JR-31 | 62 | 76 | C | E | 135 | Black gray | Medium | 80 |
| JR-33 | 56 | 72 | C | E | 95 | Light yellow | Small | 90 |
| JR-34 | 61 | 76 | C | E | 100 | Light yellow | Medium | 75 |
| JR-38 | 71 | 87 | C | E | 120 | Red-gray | Medium | 75 |
| JR-39 | 79 | 88 | C | E | 135 | Yellow | Medium | 85 |
| JR-40 | 64 | 79 | C | E | 110 | Light Brown | Medium | 80 |
| JR-42 | 59 | 83 | C | E | 95 | Light yellow | Small | 95 |
| JR-43 | 79 | 79 | C | E | 125 | Light yellow | Medium | 110 |
| JR-44 | 88 | 80 | C | E | 140 | Brown | Medium | 120 |
| JR-45 | 78 | 82 | C | E | 135 | Brown | Medium | 120 |
| JR-46 | 79 | 93 | C | E | 135 | Light Brown | Medium | 110 |
| JR-47 | 76 | 87 | C | E | 135 | Brown | Medium | 100 |
| JR-50 | 58 | 83 | C | E | 90 | Brown | Small | 85 |
| JR-53 | 58 | 86 | C | E | 90 | Light yellow | Small | 85 |
| JR-54 | 63 | 92 | C | E | 95 | Brown | Small | 90 |
| JR-57 | 64 | 98 | C | E | 95 | Yellow | Medium | 80 |
| JR-58 | 75 | 115 | C | E | 104 | Light Brown | Small | 95 |
| JR-59 | 68 | 124 | C | E | 100 | Light Brown | Medium | 85 |
| JR-60 | 67 | 115 | C | SE | 100 | Brown | Medium | 80 |
| JR-62 | 64 | 132 | C | E | 100 | Brown | Medium | 85 |
| JR-64 | 70 | 147 | C | SE | 110 | Light yellow | Medium | 90 |
| JR-65 | 69 | 134 | C | SE | 105 | Light yellow | Medium | 80 |
| JR-66 | 68 | 127 | C | SE | 105 | Yellow | Medium | 85 |
| JR-67 | 72 | 95 | C | E | 110 | Light Brown | Medium | 100 |
| JR-68 | 56 | 86 | C | E | 87 | Brown | Small | 110 |
| JR-69 | 68 | 97 | C | SE | 98 | Brown | Small | 85 |
| JR-70 | 82 | 105 | C | E | 122 | Light yellow | Medium | 95 |
| JR-71 | 65 | 89 | C | SE | 94 | Brown | Medium | 100 |
| JR-72 | 67 | 86 | C | SE | 99 | Yellow | Small | 95 |

Germplasm evaluation, AAU

| Name | Days to 50% Flowering | Plant height (cm) | Type of maturity | Growth habit | Maturity | Seed Colour | Seed size | Yield (g) |
|-----------|-----------------------|-------------------|------------------|--------------|----------|-----------------|-----------|-----------|
| JCR-07-7 | 91 | 235.00 | C | SE | 138 | Light yellow | Bold | 133.33 |
| JCR-07-15 | 97 | 98.33 | C | SE | 139 | Greenish yellow | Medium | 166.66 |
| JCR-08-1 | 84 | 171.66 | C | SE | 130 | Light yellow | small | 100.00 |
| JCR-08-2 | 90 | 190.00 | C | SE | 128 | Brown | Small | 125.00 |
| JCR-08-4 | 107 | 176.89 | C | SE | 140 | Grey | Bold | 125.00 |
| JCR-08-6 | 87 | 215.00 | C | SE | 126 | Black | Bold | 125.00 |
| JCR-08-7 | 98 | 158.75 | C | SE | 130 | Black | Bold | 333.33 |
| JCR-08-9 | 98 | 143.34 | C | SE | 139 | Brown | Bold | 433.33 |
| JCR-08-10 | 103 | 233.33 | C | SE | 139 | Light yellow | Bold | 416.66 |
| JCR-08-11 | 91 | 198.33 | C | SE | 136 | Light yellow | Small | 125.00 |
| JCR-08-12 | 106 | 245.00 | C | SE | 134 | Light yellow | Small | 166.66 |
| JCR-08-13 | 100 | 170.11 | C | SE | 136 | Light Brown | Small | 125.00 |
| JCR-08-14 | 86 | 196.66 | C | SE | 131 | Light Red | Small | 125.00 |
| JCR-08-15 | | 234.33 | C | SE | 138 | Yellow | Bold | 166.66 |
| JCR-08-16 | | 108.33 | C | SE | 128 | Grey | Bold | 125.00 |
| JCR-08-17 | 96 | 139.00 | C | SE | 140 | Light yellow | Small | 522.22 |
| JCR-08-19 | 88 | 235.00 | C | SE | 135 | Brown | Small | 166.66 |
| JCR-08-22 | | 213.33 | C | SE | 125 | Grey | Bold | 125.00 |
| JCR-08-24 | 95 | 101.33 | C | SE | 131 | Brown | Bold | 100.00 |
| JCR-08-25 | 97 | 155.00 | C | SE | 136 | Grey | Small | 125.00 |
| JCR-08-27 | 85 | 226.66 | C | SE | 139 | Yellow | Bold | 166.66 |
| JCR-08-29 | 87 | 283.33 | C | SE | 132 | Light yellow | Small | 125.00 |
| JCR-08-30 | 80 | 206.66 | C | SE | 128 | Yellow | Small | 100.00 |
| JCR-08-31 | 85 | 243.33 | C | SE | 135 | Light yellow | Small | 250.00 |
| JCR-08-32 | 88 | 213.33 | C | SE | 134 | Light yellow | Small | 416.66 |
| JCR-08-33 | 87 | 146.85 | C | SE | 128 | Brown | Big | 183.00 |
| JCR-08-34 | 90 | 135.25 | C | SE | 135 | Light Red | Small | 100.00 |
| JCR-08-35 | | 122.00 | C | SE | 138 | Grey | Bold | 116.66 |
| JCR-08-37 | 93 | 215.00 | C | SE | 132 | Light yellow | Bold | 125.00 |
| JCR-08-40 | 91 | 256.66 | C | SE | 129 | Light yellow | Small | 333.33 |
| JCR-08-41 | 90 | 169.59 | C | SE | 126 | Light yellow | Bold | 500.00 |
| JCR-08-43 | | 148.66 | C | SE | 135 | Yellow | Medium | 125.00 |
| JCR-08-45 | 88 | 188.33 | C | SE | 129 | Brown | Small | 183.33 |
| JCR-08-46 | 93 | 181.66 | C | SE | 135 | Black | Small | 125.00 |
| JCR-08-49 | 93 | 151.66 | C | SE | 131 | Light yellow | Bold | 250.00 |
| JCR-08-50 | 92 | 138.33 | C | SE | 140 | Light yellow | Bold | 183.33 |
| LRB-158 | 95 | 73.33 | C | SE | 132 | Grey | Small | 125.00 |
| LRB-5 | 91 | 96.83 | C | SE | 129 | Brown | Bold | 125.00 |
| LRB-260 | 93 | 90.00 | C | SE | 129 | Grey | Bold | 166.66 |
| LRB-31 | 100 | 92.16 | C | SE | 139 | Grey | Small | 125.00 |
| LRB-65 | 96 | 30.00 | C | SE | 141 | Grey | Small | 125.00 |
| LRB-32 | 102 | 180.66 | C | SE | 135 | Yellow | Small | 112.00 |
| LRB-160 | 99 | 86.66 | C | SE | 138 | Grey | Bold | 150.00 |
| LRB-57 | 98 | 73.33 | C | SE | 125 | Brown | Bold | 150.00 |
| LRB-55 | 87 | 76.90 | C | SE | 135 | Brown | Big | 125.00 |
| LRB-71-3 | 92 | 101.66 | C | SE | 130 | Grey | Bold | 150.00 |
| NRB-3 | 97 | 93.33 | C | SE | 131 | Brown | Bold | 175.00 |
| LRB-21 | 102 | 146.32 | C | SE | 149 | Yellow | Small | 100.00 |
| LRB-164 | 99 | 98.66 | C | SE | 139 | Yellow | Bold | 125.00 |

| Name | Days to 50% Flowering | Plant height (cm) | Type of maturity | Growth habit | Maturity | Seed Colour | Seed size | Yield (g) |
|---------|-----------------------|-------------------|------------------|--------------|----------|-------------|-----------|-----------|
| LRB-254 | 87 | 96.66 | C | SE | 134 | Grey | Bold | 150.00 |
| LRB-193 | 93 | 80.00 | C | SE | 134 | Brown | Bold | 140.00 |
| NRB-14 | 95 | 105.00 | C | SE | 138 | Brown | Small | 125.00 |
| LRB-54 | 105 | 132.20 | C | SE | 133 | Yellow | Bold | 175.00 |
| LRB-4 | 100 | 139.88 | C | SE | 135 | Brown | Bold | 150.00 |
| LRB-264 | 98 | 116.66 | C | SE | 140 | Grey | Bold | 125.00 |
| LRB-148 | 85 | 96.66 | C | SE | 125 | Yellow | Small | 116.66 |
| LRB-28 | 87 | 100.00 | C | SE | 115 | Brown | Bold | 166.66 |
| LRB-23 | 92 | 85.33 | C | SE | 138 | Brown | Small | 125.00 |
| LRB-34 | 94 | 145.35 | C | SE | 128 | Yellow | Small | 116.66 |
| LRB-31 | 97 | 92.16 | C | SE | 131 | Grey | Small | 100.00 |
| LRB-27 | 87 | 54.80 | C | SE | 129 | Grey | Bold | 125.00 |
| LRB-61 | 93 | 55.00 | C | SE | 138 | Yellow | Small | 140.00 |
| LRB-48 | 97 | 100.00 | C | SE | 137 | Brown | Bold | 125.00 |
| LRB-239 | 101 | 70.00 | C | SE | 145 | Brown | Bold | 175.00 |
| LRB-113 | 96 | 81.83 | C | SE | 138 | Brown | Small | 100.00 |
| NRB-14 | | 105.00 | C | SE | 136 | Brown | Bold | 125.00 |
| JCR-46 | | 171.25 | C | SE | 140 | Yellow | Medium | 125.00 |
| LRB-53 | | 172.33 | C | SE | 138 | Yellow | Bold | 150.00 |
| LRB-191 | | 88.33 | C | SE | 136 | Brown | Small | 125.00 |
| LRB-13 | | 79.65 | C | SE | 132 | Yellow | Small | 150.00 |
| LRB-1NE | | 179.33 | C | SE | 130 | Green | Bold | 175.00 |
| LRB-193 | | 93.33 | C | SE | 135 | Brown | Bold | 125.00 |
| LRB-148 | | 94.25 | C | SE | 140 | Yellow | Small | 150.00 |
| LRB-27 | | 59.95 | C | SE | 141 | Grey | Bold | 100.00 |
| LRB-51 | | 203.33 | C | SE | 137 | Yellow | Small | 175.00 |
| JCR-51 | 93 | 224.33 | C | SE | 139 | Yellow | Small | 166.66 |
| JCR-52 | 94 | 144.66 | C | SE | 140 | Red | Medium | 250.00 |
| JCR-53 | 95 | 242.33 | C | SE | 138 | Yellow | Medium | 216.66 |
| JCR-54 | 97 | 141.00 | C | SE | 138 | Grey | Bold | 166.66 |
| JCR-55 | | 259.00 | C | SE | 140 | Yellow | Medium | 416.00 |

Annex 2. Promising lines of ricebean for different traits, India, 2009

| Characters | Promising lines |
|------------------------------------|--|
| Days to 50% flowering | RBHP- 2, RBHP- 3, RBHP- 7, RBHP- 8, RBHP- 9, RBHP- 10, RBHP- 12, RBHP- 13, RBHP- 22, RBHP- 24, RBHP- 26, RBHP- 30, RBHP- 36, RBHP- 43, RBHP- 44b , RBHP- 45, RBHP- 59, RBHP- 62a, RBHP- 63, RBHP- 65, RBHP- 66, RBHP- 72, RBHP- 74, RBHP- 75, RBHP- 78, RBHP- 80, RBHP- 82, RBHP- 86, RBHP- 89, RBHP- 95, RBHP- 99, RBHP- 100, RBHP- 110, RBHP- 112, , , |
| Flowering period | RBHP-5, RBHP-23, RBHP -14, RBHP -20 , RBHP -49, RBHP-62(B), RBHP -75, RBHP – 77, RBHP - 79, RBHP – 81, RBHP – 83 RBHP – 85, RBHP – 87, RBHP-88, RBHP – 94, RBHP -95 , RBHP -96 ,RBHP – 101, RBHP – 102 , RBHP -103, RBHP -107, RBHP -108 , RBHP -109, RBHP -110 |
| Days to first mature pod | RBHP-6, RBHP-18, RBHP-26, RBHP- 30, RBHP- 36, RBHP- 41, RBHP- 44b, RBHP- 44c, RBHP- 43, RBHP- 45, RBHP- 55, RBHP- 56, RBHP- 62a , RBHP-63, RBHP- 72, RBHP- 73, RBHP- 97, RBHP- 100 |
| Terminal leaflet blade length (cm) | RBHP- 2, RBHP- 7, RBHP- 13, RBHP- 26, RBHP- 30, RBHP- 37, RBHP- 38, RBHP- 50, RBHP- 51, RBHP- 61, RBHP- 63, RBHP- 64, RBHP- 66, RBHP- 69, RBHP- 71, RBHP- 72, RBHP- 83, RBHP- 85, RBHP- 86, RBHP- 87, RBHP- 88, RBHP- 89, RBHP- 93, RBHP- 97, RBHP- 99, , RBHP- 101, RBHP- 112 |
| Terminal leaflet blade width (cm) | RBHP- 2, RBHP- 3, RBHP- 7 , RBHP- 12, RBHP- 13, RBHP- 35, RBHP- 44b, RBHP- 50, RBHP- 54, RBHP- 61, RBHP- 63, RBHP- 64, RBHP- 66, RBHP- 71, RBHP- 72, RBHP- 85, RBHP- 87, RBHP- 97, RBHP- 101, RBHP- 102, RBHP- 112 |
| Plant height (cm) | RBHP -1, RBHP -4, RBHP-5, RBHP -6, RBHP-16, RBHP -18, RBHP-19, RBHP -20, RBHP-23, RBHP -24, RBHP -25, RBHP -27, RBHP – 29, RBHP – 30, RBHP – 32, RBHP – 46, RBHP-62(B), RBHP – 66, RBHP-70, RBHP -75, RBHP – 76, RBHP – 77, RBHP – 83, RBHP -96, RBHP -106, RBHP -107, RBHP – 102 |
| Pods per plant | RBHP- 2, RBHP- 3, RBHP- 7, RBHP- 8, RBHP- 9, RBHP- 11, RBHP- 12, RBHP- 26, RBHP- 31, RBHP- 35, RBHP- 39, RBHP- 44b, RBHP- 51, RBHP- 53, RBHP- 54, RBHP- 56, RBHP- 67, RBHP- 71, RBHP- 78, RBHP- 81, RBHP- 82, RBHP- 86, RBHP- 87, RBHP- 94, RBHP- 99, RBHP- 109, RBHP- 113, RBHP- 114, |
| Pod length (cm) | RBHP- 37, RBHP- 38, RBHP- 41, RBHP- 43, RBHP- 44a, RBHP- 44b , RBHP- 44c, RBHP- 45, RBHP- 48, RBHP- 50, RBHP- 51, RBHP- 53, RBHP- 54, RBHP- 55, RBHP- 60, RBHP- 61, RBHP- 62a, RBHP- 63, RBHP- 64, RBHP- 66 , RBHP- 67, RBHP- 69, RBHP- 72, RBHP- 73, RBHP- 80, RBHP- 87, RBHP- 97, RBHP- 101, RBHP- 102 |
| 100-Seed weight (g) | RBHP- RBHP- 2, 10, RBHP- 12, RBHP- 23, RBHP- 31, RBHP- 36, RBHP- 41, RBHP- 44b, RBHP- 44c, RBHP- 45, RBHP- 47, RBHP- 48, RBHP- 50, RBHP- 52, RBHP- 53, RBHP- 55, RBHP- 56, RBHP- 61, RBHP- 64, RBHP- 63, RBHP- 67, RBHP- 71, RBHP- 72, RBHP- 73, RBHP- 78, RBHP- 80, RBHP- 85, RBHP- 90, RBHP- 97, RBHP- 99, RBHP- 100, RBHP- 101, RBHP- 102, RBHP- 105, RBHP- 111, RBHP- 113, RBHP- 114 |
| Seed yield per plant (t/ha.) | RBHP -2, RBHP -3, RBHP -7, RBHP-9, RBHP -10, RBHP-11, RBHP -18, RBHP -20, RBHP – 29, RBHP – 30, RBHP -39, RBHP-42, RBHP -44-A, RBHP -44(B), RBHP – 51, RBHP -53, RBHP – 63, RBHP – 64, RBHP – 66, RBHP -67 , RBHP – 72, RBHP – 76, RBHP – 85, RBHP -86, RBHP – 87, RBHP-112, RBHP -110, RBHP-113, RBHP -114 |
| Pod Duration | RBHP -1, RBHP-9, RBHP-11, RBHP-12 , RBHP – 17, RBHP -20, RBHP-23, RBHP -24, RBHP -25, RBHP -27, RBHP -39, RBHP -49, RBHP -67, RBHP-70, RBHP -74, RBHP – 76, RBHP – 77, RBHP - 78, RBHP – 81, RBHP -82, RBHP – 83, RBHP – 85, RBHP -86, RBHP – 87, RBHP – 94, RBHP – 102, RBHP -106, RBHP -107, RBHP -108, RBHP -110, RBHP-112 , RBHP-113, RBHP -114 |

Annex 3. Grouping of lines based on traits performance in germplasm trials:

| Trait | No. | Entries |
|----------------------------|-----|--|
| Plant height (cm) | | |
| 40- 70 | 27 | RBHP -106, RBHP-62(B), RBHP-23, RBHP-70, RBHP – 66,, RBHP -25, RBHP -74, RBHP -20, RBHP – 76, RBHP -18, RBHP-19, RBHP – 46, RBHP -96, RBHP -24, RBHP – 32, RBHP -107 RBHP-5 , RBHP – 102, RBHP – 83, RBHP – 29, RBHP -27, RBHP – 77,, RBHP – 30, RBHP-16 RBHP -6, RBHP -75, RBHP -1 |
| 71- 90 | 40 | RBHP-89,, RBHP -49, RBHP-84, RBHP-100, RBHP -114, RBHP-45, RBHP – 94, RBHP – 17, RBHP -108, RBHP – 81, RBHP -41, RBHP -14, RBHP -93, RBHP -99, RBHP-112, RBHP-90, RBHP -37 RBHP-105, RBHP -69, RBHP -104, RBHP – 87, RBHP -60, RBHP – 72, RBHP-80, RBHP -67 RBHP-73, RBHP -97, RBHP-35, RBHP -59, RBHP – 47, RBHP-42, RBHP -53, RBHP-44C RBHP – 22, RBHP-113, RBHP – 36, RBHP -79,, RBHP -39, BRS II, RBHP-71 |
| 91-110. | 29 | RBHP -48, RBHP – 85, RBHP -103, RBHP -38, RBHP-88, RBHP -62(A),, RBHP -44-A, RBHP -52 RBHP-31, RBHP – 65, RBHP-56, RBHP -109, RBHP -82, RBHP – 50, RBHP-9, Nainy , RBHP-11 RBHP – 13, RBHP -110, RBHP -95, RBHP -8, RBHP – 63, RBHP – 61, RBHP – 43, RBHP -101 RBHP -26, RBHP -10, RBHP -78 |
| >110 | 12 | RBHP -58, RBHP-12, RBHP -2, RBHP -111, RBHP -44(B), RBHP -55, RBHP – 51, RBHP -54, RBHP – 64, RBHP -86, RBHP -7, RBHP -3 |
| Flowering Period | | |
| 25 to 35 | 46 | RBHP – 81, RBHP-23, RBHP -14, RBHP – 94, RBHP -79, RBHP -103, RBHP -109, RBHP-62(B), RBHP -49, RBHP -110, RBHP – 85, RBHP -95, RBHP -107, RBHP -20, RBHP -108, RBHP -75, RBHP – 87, RBHP – 101, RBHP – 102, RBHP -96, RBHP – 77, RBHP – 83, RBHP-88, RBHP-5, RBHP -82, RBHP – 51, RBHP -37, RBHP -104, RBHP – 76, RBHP-71, RBHP-90, RBHP -67, RBHP – 66, RBHP -10, RBHP-70, RBHP -25, RBHP -114, RBHP-12, RBHP -24, RBHP-42, RBHP -27, RBHP -99, RBHP -78, RBHP -39, RBHP – 17, RBHP-112 |
| 36 to 45 | 33 | RBHP – 64, RBHP – 29, RBHP – 30, RBHP – 46, RBHP – 22, RBHP -1, RBHP -74, RBHP-84, RBHP-11, RBHP-9, RBHP -111, BRS I , RBHP -69, RBHP-19, RBHP -106, RBHP -44-A, RBHP-105, RBHP -86, RBHP -93, RBHP-89, RBHP – 72, RBHP -59, RBHP – 61, RBHP-113, RBHP – 47, RBHP -55, RBHP – 50, RBHP – 52, RBHP – 32, RBHP-31, RBHP-44C, RBHP-100, RBHP -60 |
| > 46 | 29 | RBHP – 36, RBHP-73, RBHP-80, RBHP -53 , RBHP -6, RBHP – 65, RBHP-16, RBHP -18, RBHP -7, RBHP -26, RBHP – 63, RBHP-56, RBHP-35, RBHP -97, RBHP -48, RBHP -2 , Nainy, RBHP – 13, RBHP -41, RBHP -3, BRS II, RBHP -58, RBHP -44(B), RBHP – 43, RBHP -62(A), RBHP -54, RBHP-45, RBHP -38, RBHP -8 |
| Pod duration (days) | | |
| 13 to 20 | 33 | RBHP – 76, RBHP – 85, RBHP – 81, RBHP -49, RBHP -114, RBHP – 17, RBHP -24, RBHP-112 RBHP-70, RBHP -107, RBHP -74, RBHP -108, RBHP – 87, RBHP – 77, RBHP – 94, RBHP -27 RBHP -1, RBHP -78, RBHP-9, RBHP – 83, RBHP -25, RBHP -82, RBHP -39, RBHP-113, RBHP-12 RBHP – 102, RBHP -106, RBHP -20, RBHP -67, RBHP -86, RBHP-11, RBHP-23, RBHP -110 |
| 21 to 30 | 47 | RBHP -14, RBHP -3 , RBHP – 29, RBHP – 22, RBHP -99, RBHP -8, RBHP -109, RBHP -93, RBHP -111, RBHP – 64, RBHP – 66, RBHP – 32, RBHP – 52, RBHP -96, RBHP -59, RBHP |

| | | |
|---------------------------|----|--|
| | | -103 RBHP -95, RBHP -44-A, RBHP-89, RBHP – 46, RBHP – 37, RBHP-16, RBHP -2, RBHP - 51 RBHP -79, RBHP-42, RBHP-5 , RBHP-90, RBHP-62(B), RBHP -7, RBHP – 30, RBHP – 50, RBHP -10, RBHP -55, RBHP – 72, RBHP -104, RBHP – 61, BRS I, RBHP-88, RBHP – 65, RBHP - 36 RBHP -69, RBHP -6, RBHP – 47, RBHP – 13, RBHP-100, RBHP -58, |
| > 31 | 28 | RBHP -18, RBHP -48, RBHP - 43, RBHP-19, RBHP-45, RBHP -97, RBHP -60, RBHP -62(A) RBHP-84, RBHP -44(B), RBHP – 63, RBHP-80, RBHP -54, RBHP -53, RBHP -26, BRS II RBHP-44C, Nainy, RBHP -41, RBHP – 101, RBHP-71, RBHP-73, RBHP -38, RBHP-31, RBHP- 105 RBHP-35, RBHP-56, RBHP -75 |
| Grain yield (t/ha) | | |
| 0.32 -0.80 | 65 | RBHP-105, RBHP-62(B), RBHP – 83, RBHP – 101, RBHP -96, RBHP – 81, RBHP -14, RBHP-5 RBHP-90, RBHP -111, RBHP – 47, RBHP -107, RBHP-35, RBHP – 32, RBHP-45, RBHP - 108, RBHP-88 RBHP -49, RBHP -104, RBHP-56, RBHP-73, RBHP – 65, RBHP-44C, RBHP -27, RBHP- 84, RBHP -25, RBHP-80, RBHP-100, RBHP -6, RBHP -58, RBHP – 37, RBHP – 94, RBHP – 61, RBHP -62(A), RBHP -103, RBHP -106, RBHP – 77, RBHP -109, RBHP – 102, RBHP- 19, RBHP – 46, RBHP – 22, RBHP-71. RBHP -24, RBHP -69, Nainy, RBHP-23, BRS-II, RBHP - 1, RBHP-89, RBHP-31, RBHP -48, RBHP -82, RBHP -95, RBHP-70, RBHP -8, RBHP -97, RBHP -55, RBHP -79, RBHP-16, RBHP - 50 RBHP -74, RBHP -60, RBHP-12, RBHP -93 |
| 0.81-1.03 | 26 | RBHP - 13 RBHP - 52 RBHP -59 RBHP -75 RBHP - 17 RBHP - 36 RBHP -78 RBHP -99 RBHP -54 RBHP -18 RBHP -26 RBHP -39 RBHP -114 RBHP -10 RBHP-113 RBHP -44(B) RBHP - 87 RBHP - 64 RBHP - 30 RBHP - 63 RBHP - 85 RBHP -67 RBHP-11 RBHP -20 RBHP-9 RBHP -110 |
| > 1.04 | 17 | BRS-I RBHP-42 RBHP -41 RBHP -44-A RBHP -53 RBHP - 51 RBHP - 66 RBHP -38 RBHP - 72 RBHP -2 RBHP - 29 RBHP - 76 RBHP - 43 RBHP-112 RBHP -3 RBHP -86 RBHP -7 |

Annex 4: Molecular diversity assay in selected stratified random samples of rice bean 2009

(A protocol)

Background and introduction

A tremendous diversity of ricebean at various levels in plant type, seed morphologies and use values has been found under cultivation. It is adapted to range of altitudes from humid subtropical to warm and cool temperate regions of diverse agro-ecosystems from lowland to high-hills of Nepal. Its cultivation extends from east to western limits of the country. It is an important food legume particularly for mid-hills of Nepal with a pivotal role as pulse in dietary components supporting the food security of the rural poor in mid-hills. A good collection of rice bean germplasm is held with NARC genebank as landrace accessions collected by Agriculture Botany Division (ABD), the Plant Genetic Resource Unit (PGRU) from different districts of the country. In addition, FOSRIN on its implementation have collected 156 accessions from 16 districts in 2006. These germplasm under evaluation and characterization using agro-morphological characters have shown a wide variation and a set of information on agro-morphological characterization of these accessions have been generated. However, for exploitation of germplasm in breeding programme, it is always valuable and good to generate the molecular data on genetic diversity. Knowledge on genetic diversity among these valuable resources is therefore important for effective conservation and success of a breeding programme. With this the WP3 has been foreseen in the project. Under this work package there has screened out 109 SSR primer pairs of adzuki bean on a stratified random samples of rice bean and have detected 35 primers polymorphic with 2-4 alleles per locus. Likewise, a comprehensive collection of landraces have been selected for this SSR diversity. The selection of accessions is based to information on agro-morphological and physic-graphical distribution of diversity.

Objective

- to evaluate rice bean germplasm for molecular marker diversity and understand the relationship of molecular and agro-morphological diversity in rice bean of Nepal

Materials

- All 50 accessions under core collection field evaluation in 2007/08 in 4 different environments
- Stratified selection of 41 accessions under non-core collection field evaluation in 2007/08 in 4 different environments
- Adjuki bean (*Vigna angularis*) as check sample for primers
- Bold seeded, climbing rice bean from Illam (*Vigna umbellata*) as check sample for rice bean samples under study
- 25 accessions randomly selected (12 + 12 + 1 Assam, Himanchal Pradesh and Gujarat)
- In total there will be (50 + 41+ 25) + 2 checks

Details of those landraces selected for the study are provided in Table 1.

Methodology/process/steps

Sampling

- Selection of the landraces for the study is based on the distribution and the agro-morphological diversity (Table 1).
- Criteria for selection of random samples from non-core collection was the aggregate values of PC1 and PC2 for qualitative and quantitative traits together, which was then sorted in EXCEL from descending to ascending values and picked one accession for every fourth from a total of 164 accessions. It is done with the idea to capture the diversity in totality.

SSR primers

- Thirty five polymorphic SSR primers will be used for microsatellite analysis on these samples. Sequence and motifs of these polymorphic SSR primers are given in Table 2.

DNA isolation

- Phytopure Genomic dna extraction kit will be used
- Bulk DNA of 15 seeds of each accession under this study will be used.
- A total of 91 DNA samples of rice bean landraces along with 2 checks will be tested for to the maximum 35 polymorphic SSR primers.
- Based to diversity analysis using 35 poly primers, individual DNAs of most diverse genotypes will be further analysed for diversity within population

Thermal cycling and electrophoresis

- PCR volume will be 20 µl contained with 4-5 ng of genomic DNA, 10 µl of Reddy Mix™ PCR Master Mix contained of 3.0 mM MgCl₂; 10xPCR buffer, Taq polymerase and blue dye (ABgene, Epsom, Surrey, UK) and 20 µM of forward and reverse primers.
- The PCR thermal cycling programme to be used will be of initial denaturation at 94°C for 2 mins followed by 35 cycles of: denaturation at 94°C for 30 secs, annealing at 50°C for 30 secs and elongation at 68°C for 30 secs followed by further elongation at 68°C for 2 mins and finally to hold at 4°C for infinite.
- Amplified products will be checked by electrophoresis in 3 % high resolution Amresco SFR agarose gel at constant voltage of 90 for 4 hrs.

Statistical analysis

- Banding pattern and size documentation will be carried under bio-doc gel analyzer and binary data matrix will be generated.
- Diversity parameters like allelic richness, number of alleles per locus, frequencies of alleles, heterozygosity level, genetic relationship and diversity indices will be calculated based on the size, number and distance travelled by the bands.
- Agro-morphological diversity and molecular diversity will be compared and relate this diversity with biophysical and socio-economic values of rice bean germplasm
- NTSYS pc version 2.02; excel and others related tools will be used.

Results

- Evaluation of stratified sample of ricebean with identified markers
- Analysis of molecular marker data
- Comparison of molecular data with agro-morphological data

Project deliverables

- Ricebean diversity in Nepal and India described on the basis of molecular markers (D3.2)
- The value of diversity estimates using agro-morphological or molecular markers described in relation to biophysical and socio-economic variables (D3.3)

Table 1: List of selected accession for SSR diversity assay

| S. No | Accession number | Collection districts | Source | Remarks |
|-------|------------------|----------------------|---------------------|----------------------------------|
| 1 | NPGR-00007 | Nuwakot, Nepal | Core collection | |
| 2 | NPGR-00008 | Nuwakot, Nepal | Core collection | Included in mother trial in 2008 |
| 3 | NPGR-00010 | Lalitpur, Nepal | Core collection | |
| 4 | NPGR-00012 | Nuwakot, RBHP | Core collection | |
| 5 | NPGR-00015 | Bhaktapur, Nepal | Core collection | Included in mother trial in 2008 |
| 6 | NPGR-00073 | Gulmi, Nepal | Core collection | |
| 7 | NPGR-00076 | Arghakhanchi, Nepal | Core collection | Included in mother trial in 2008 |
| 8 | NPGR-00087 | Pyuthan, Nepal | Core collection | |
| 9 | NPGR-00090 | Dang, Nepal | Core collection | |
| 10 | NPGR-00194 | Kabre, Nepal | Core collection | Included in mother trial in 2008 |
| 11 | NPGR-01975 | Baitadi, Nepal | Core collection | |
| 12 | NPGR-05364 | Bhojpur, Nepal | Core collection | Included in mother trial in 2008 |
| 13 | NPGR-05368 | Bhojpur, Nepal | Core collection | |
| 14 | NPGR-05370 | Terhathum, Nepal | Core collection | |
| 15 | NPGR-05373 | Gorkha, Nepal | Core collection | |
| 16 | NPGR-05377 | Lamjung, Nepal | Core collection | |
| 17 | NPGR-05382 | Tanahu, Nepal | Core collection | |
| 18 | NPGR-05384 | Mugu, Nepal | Core collection | |
| 19 | NPGR-05386 | Humla, Nepal | Core collection | |
| 20 | NPGR-05391 | Bajura, Nepal | Core collection | |
| 21 | NPGR-05396 | Illam, Nepal | Core collection | |
| 22 | NPGR-05420 | Dhankuta, Nepal | Core collection | Included in mother trial in 2008 |
| 23 | NPGR-05423 | Dhankuta, Nepal | Core collection | |
| 24 | NPGR-05432 | Baitadi, Nepal | Core collection | |
| 25 | NPGR-05565 | Okhaldhunga, Nepal | Core collection | |
| 26 | NPGR-06591 | Mugu, Nepal | Core collection | |
| 27 | NPGR-06657 | Kalikot, Nepal | Core collection | |
| 28 | NPGR-06756 | Humla, Nepal | Core collection | Included in mother trial in 2008 |
| 29 | NPGR-07583 | Jhapa, Nepal | Core collection | |
| 30 | NPGR-07882 | Bajhang, Nepal | Core collection | |
| 31 | NPGR-08380 | Myagdi, Nepal | Core collection | |
| 32 | NPGR-08382 | Banglung, Nepal | Core collection | |
| 33 | NPGR-09391 | Syangja, Nepal | Core collection | |
| 34 | NPGR-09461 | Panchthar, Nepal | Core collection | |
| 35 | NPGR-09464 | Taplejung, Nepal | Core collection | |
| 36 | LRGR-42 | Surkhet, Nepal | Core collection | |
| 37 | LRGR-43 | Surkhet, Nepal | Core collection | |
| 38 | LRGR-44 | Surkhet, Nepal | Core collection | |
| 39 | LRGR-75 | Pyuthan, Nepal | Core collection | |
| 40 | LRGR-91 | Dang, Nepal | Core collection | Included in mother trial in 2008 |
| 41 | LRGR-99 | Palpa, Nepal | Core collection | Included in mother trial in 2008 |
| 42 | LRGR-101 | Palpa, Nepal | Core collection | |
| 43 | LRGR-102 | Palpa, Nepal | Core collection | |
| 44 | LRGR-103 | Palpa, Nepal | Core collection | Included in mother trial in 2008 |
| 45 | LRGR-107 | Palpa, Nepal | Core collection | |
| 46 | LRGR-111 | Gulmi, Nepal | Core collection | Included in mother trial in 2008 |
| 47 | LRGR-117 | Gulmi, Nepal | Core collection | Included in mother trial in 2008 |
| 48 | LRGR-129 | Palpa, Nepal | Core collection | |
| 49 | LRGR-137 | Kaski, Nepal | Core collection | |
| 50 | LRGR-152 | Kavre, Nepal | Core collection | |
| 51 | NPGR-05435 | | Non-core collection | |
| 52 | LRGR-7 | Dadeldhura, Nepal | Non-core collection | |
| 53 | NPGR-06725 | Humla, Nepal | Non-core collection | |
| 54 | LRGR-55 | Baitadi, Nepal | Non-core collection | |
| 55 | LRGR-30 | Bajura, Nepal | | |
| 56 | LRGR-8 | Dadeldhura, Nepal | Non-core collection | |
| 57 | LRGR-3 | Dadeldhura, Nepal | Non-core collection | |
| 58 | LRGR-79 | Dang, Nepal | Non-core collection | |
| 59 | NPGR-00014 | Nuwakot, Nepal | Non-core collection | |
| 60 | FOSRIN-06 | Kathmandu, Nepal | Non-core collection | |
| 61 | NPGR-00183 | Kavre, Nepal | Non-core collection | |
| 62 | FOSRIN-02 | Dailekh, Nepal | Non-core collection | |
| 63 | LRGR-90 | Dang, Nepal | Non-core collection | |

| S. No | Accession number | Collection districts | Source | Remarks |
|-------|------------------|----------------------|---------------------|----------------------------|
| 64 | LRGR-132 | Gulmi | Non-core collection | |
| 65 | NPGR-00091 | Dang | Non-core collection | |
| 66 | NPGR-00088 | Pyuthan | Non-core collection | |
| 67 | NPGR-00188 | Kavre | Non-core collection | |
| 68 | NPGR-05395 | Unknown | Non-core collection | |
| 69 | NPGR-05367 | Bhojpur | Non-core collection | Included in OBN in 2008 |
| 70 | NPGR-05424 | Dhankuta | Non-core collection | Included in OBN in 2008 |
| 71 | LRGR-133 | Palpa | Non-core collection | Included in OBN in 2008 |
| 72 | NPGR-00199 | Kabhre | Non-core collection | |
| 73 | LRGR-148 | Kavre | Non-core collection | |
| 74 | NPGR-05404 | Illam | Non-core collection | |
| 75 | NPGR-00074 | Arghakhanchi | Non-core collection | |
| 76 | LRGR-151 | Kavre | Non-core collection | |
| 77 | LRGR-123 | Palpa | Non-core collection | Included in OBN in 2008 |
| 78 | NPGR-05380 | Lamjung | Non-core collection | |
| 79 | LRGR-118 | Gulmi | Non-core collection | |
| 80 | NPGR-05383 | Tanahun | Non-core collection | |
| 81 | NPGR-05398 | Illam | Non-core collection | |
| 82 | NPGR-05372 | Gorkha | Non-core collection | Included in OBN in 2008 |
| 83 | NPGR-05399 | Illam | Non-core collection | |
| 84 | LRGR-146 | Kaski | Non-core collection | |
| 85 | NPGR-05409 | Illam | Non-core collection | |
| 86 | NPGR-05401 | Illam | Non-core collection | |
| 87 | LRGR-73 | Pyuthan | Non-core collection | |
| 88 | LRGR-145 | Kaski | Non-core collection | |
| 89 | NPGR-05410 | Dhankuta | Non-core collection | |
| 90 | LRGR-159 | Kaski | Non-core collection | |
| 91 | LRGR-135 | Palpa | Non-core collection | |
| 92 | JCR-08-2 | Assam, India | Agro-morphology | |
| 93 | JCR-08-10 | Assam, India | Agro-morphology | |
| 94 | JCR-08-12 | Assam, India | Agro-morphology | |
| 95 | JCR-08-15 | Assam, India | Agro-morphology | |
| 96 | JCR-08-27 | Assam, India | Agro-morphology | |
| 97 | JCR-08-30 | Assam, India | Agro-morphology | |
| 98 | JCR-08-35 | Assam, India | Agro-morphology | |
| 99 | JCR-08-45 | Assam, India | Agro-morphology | |
| 100 | JCR-08-50 | Assam, India | | Not included |
| 101 | JCR-08-52 | Assam, India | | Not included |
| 102 | JCR-08-53 | Assam, India | Agro=morphology | |
| 103 | Shyamalee | Assam, India | Agro-morphology | |
| 104 | JR-1 | Gujarat, India | Agro-morphology | |
| 105 | RBHP-7 | Palampur, India | Agro | |
| 106 | RBHP-16 | Palampur, India | Agro | |
| 107 | RBHP-26 | Palampur, India | Agro | |
| 108 | RBHP-28 | Palampur, India | Agro | |
| 109 | RBHP-32 | Palampur, India | Agro | |
| 110 | RBHP-42 | Palampur, India | Agro | |
| 111 | RBHP-52 | Palampur, India | Agro | |
| 112 | RBHP-60 | Palampur, India | Agro | |
| 113 | RBHP-65 | Palampur, India | Agro | |
| 114 | RBHP-76 | Palampur, India | Agro | |
| 115 | RBHP-79 | Palampur, India | | Not included |
| 116 | RBHP-94 | Palampur, India | | Not included |
| 117 | Adzuki bean | Bangor | Optimization/(PCR) | Dr P A Hollington provided |
| 118 | Rice bean | Illam | Optimization/(PCR) | Dr P Anderson collected |
| 119 | Gurans | Baitadi | Optimization (PCR) | |

Table 2: List of polymorphic SSR primers of Adzuki bean screened out

| Primers | Linkage | Forward Primer (5'-3') | Reverse Primer (5'-3') | Motif |
|-----------|---------|--------------------------|--------------------------|-----------------|
| CEDG127 | 4 | GGTTAGCATCTGAGCTTCTTCGTC | CTCCTCACTTGGTCTGAAACTC | (TG)3(AG)9 |
| CEDG018 | 5 | AGCGTGTTTGTGGTGATAGC | ACACAGGAACGAACAAACCC | (AG)32 |
| CEDG150 | 10 | GAAGGGAATGAAAATGAAACCC | GTTCAATCCATTCAGTCTCC | (AG)14 |
| CEDG214 | 5 | CACTCACTGCAAAGAGCAAC | CTACCTATCTGAGGGACAC | (AG)4 AA(AG)31 |
| CEDAAG002 | 2 | GCAGCAACGCACAGTTTCATGG | GCAAAACTTTTCACCGGTACGACC | (AAG)16 |
| CEDG204 | 7 | CCTTGGTTGGAGCAGCAGC | CACAGACACCCTCGCATG | (AG)15 |
| CEDG043 | 3 | AGGATTGTGGTTGGTGATG | ACTATTTCCAACCTGCTGGG | (AG)14 |
| CEDG021 | 10 | GCAGAATTTTAGCCACCGAG | AAAGGATGCGAGAGTGTAGC | (AG)26 |
| CEDG084 | 3 | ATCAACTGAGGAGCATCATCGA | CAACATTTCAACCTTGGGACAG | (AG)13 |
| CEDG015 | 1 | CCCAGTGAACGCTAATGCTG | CGCCAAAGGAAACGCAGAAC | (AG)27 |
| CEDG026 | 2 | TCAGCAATCACTCATGTGGG | TGGGACAAACCTCATGGTTG | (AG)26 |
| CEDG073 | 8 | CCCCGAAATTCCTTACAC | AACACCCGCCTCTTTCTCC | (AG)24 |
| CEDG007 | | | | |
| CEDG008 | 5 | AGGCGAGGTTTCGTTTCAAG | GCCCATATTTTACGCCAC | (AG)26 |
| CEDG286 | 2 | CGAGCAGAACACTGATCATG | CCTCTTAGAGGTCATTGCTC | (AG)23 |
| CEDG294 | 3 | CACCTTCTTAATCTCTTACC | GGGTTTCTCTTAATTCATTGAGTC | (AT)27(AG)15 |
| CEDG232 | 9 | GATGACCAAGGTAACGTG | GGACAGATCCAAAACGTG | (AG)16 |
| CEDG071 | 8 | GGTCCATTGAGACGGATCGAG | TCCCACCTCAGCGGAATCC | (AG)9 |
| CEDG253 | 8 | CACTTCCATGATGACTACC | CACCCTTCTTATCCTCTTCG | (AG)30 |
| CEDG090 | 1 | ATAAGTAGAAATTGGTTCAAATG | GGTTCGTTAAAGTAACTTTAAT | (AG)28 |
| CEDG044 | 11 | TCAGCAACCTTGCAATTGCAG | TTCCCGTCACTCTTCTAGG | (GT)10 AT(AG)18 |
| CEDG141 | 1 | CCAGGCATCCATGATGACC | GAAGTTGTTGGTAATGGTTGCCTC | (AT)6(AG)13 |
| CEDG178 | 1 | CGGAAGAAGAACGCAGAGTG | GCATCAACAAGGACTTCTGC | (AG)10 G(AG)5 |
| CEDG118 | 6 | AACCCAACCAACCTTGTGGTAAG | GCTGGAATCATAATACCGCCTGT | (AG)21 |
| CEDG154 | 4 | GTCCTTGTTTTCTCTCCATGG | CATCAGCTGTTCAACACCCTGTG | (AG)14 |
| CEDG037 | 6 | GAAGAAGAACCCTACCACAG | CACCAAAAACGTTCCCTCAG | (AG)16 AC(AG)8 |
| CEDG195 | 6 | GAGGGTCTCCACTTTTGAAACCC | GATACTAAGGCTTCTCCACCCAC | (AG)11 |
| CEDG134 | 10 | CTCCGTGTTGAAACAATGACG | GGTCTTCTGATCTACGAACTTG | (AG)11 |
| CEDG104 | 11 | TATGGCCCGAGCAAACCTTG | CCGTTCGGTCTTCGGTTGAA | (AG)13 |
| CEDG050 | 2 | GGCAGAATCGTACAAGTG | GTCAGATTCTCGCTTGCATG | (AG)12 |

Annex 5: Study on date of planting for ricebean in post rainy season on-farm (2009)

(A Protocol)

Introduction

Ten to twelve best performed accessions in agro-morphological characterization of core collection were tested for yield potential traits under mother trials on on-farm/station in Dhading, Chitwan, Gulmi and Tanahun, Lalitpur and Dolakha districts representing different agro-environments in 2008. On-stations, the yield trial were carried in ABD Khumaltar, Lalitpur; NGLRP, Rampur, Chitwan and HCRP, Kabre, Dolakha. Grain yield of these genotypes across the sites was statistically analysed and genotypes above the average yield were ranked. The genotypes were then compared for yield performance across the sites and based on matrix of genotypes ranking, 4 genotypes: LRGR 91 (Dang), LRGR 111 (Gulmi), NPGR 05364 (Bhojpur), and NPGR 00008 (Nuwakot) were selected and further evaluated for yield trait in different altitudes up to 1200 m in post rainy season.

Objectives:

- to evaluate the selected genotypes for yield further in different altitudes and in post rainy season
- to identify genotypes suitable for *ghaiya* rice or maize based cropping pattern

Expected output:

Suitable genotypes with desired yield potential will be identified for upland conditions

Methodology

| Particulars | Trial details |
|---|--|
| Number of accessions | 4 accessions from mother trial (LRGR 91, LRGR 111, NPGR 00008 and NPGR 05364)) |
| Number of trials and experiment sites | 4 experiments on on-farm: <ul style="list-style-type: none"> • Chitwan (300m) – NGLRP, Rampur • Tanahun (600m) – LIBIRD, Pokhara • Dolakha (Namdu, 900m) – HCRP, Kabre • Kaski/Dhankuta (respective command site, 1200m) – LAC/PAC |
| Date of planting and time interval | 01, 15 and 30 August, 2009 (15 day intervals) |
| Trial design | RCBD with 4 replications |
| Plot size (6 rows) | 6 m ² (3 x 2) per plot |
| Spacing | 50 x 20 cm (6 rows of 2 m length) under sole cropping |
| Target cropping pattern | <i>Ghaiya</i> rice or maize based cropping lands |
| Land preparation, manures and fertilizers | As per farmers practice. (Record the quantity of FYM or compost used). Do not apply chemical fertilizers |
| Plant Population per plot | 48 plants/plot and use middle 4 rows for yield observation |
| Seed rate | Two seeds per hill but retain only single plant after full establishment in case of ricebean |
| Intercultural operations | weeding and earthing up as and when required No need for any other intercultural operations Staking provided as per need |
| Observation to be taken | Flowering and maturity Total biomass yield and grain yield |
| 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 analysis will be carried and the results will be used in identifying the best genotypes for upland single crop lands. |

Annex 6. Summary of winter adaptation trials, Jhapa, 2009/010

| Particulars | Trial details |
|--|--|
| Number of accessions | 12 accessions* |
| Experimental sites | Chandradaangi-7, Shivagunj, Jhapa district |
| Date of planting | 1 st week of November, 2009 3 rd week of November, 2009 |
| Trial design | RCBD with 3 replications |
| Plot size (6 rows) | 4 m ² (2 x 2) per plot |
| Spacing | 40 x 20 cm (5 rows of 2 m length) under sole cropping |
| Target cropping pattern | Rice fallows in eastern Terai of Nepal |
| Land preparation, manures and fertilizers | As per farmers practice. (Record the quantity of FYM or compost used). Do not apply chemical fertilizers. |
| Plant Population per plot | 70 plants/plot |
| Seed rate | Two seeds per hill but retain only single plant after full establishment |
| Intercultural operations | Two three weeding and earthing up No need for any other intercultural operations No stakes provided |
| Observation to be taken | Days to flowering Days to maturity No of pods/plant Seeds/pod Pod length Total biomass yield and Grain yield |
| Harvesting, threshing, storage | As per the farmers practice. Avoid any chance of varietal mixtures during harvesting, threshing and storage. Sun dry seeds 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 analysis will be done to identify the most adaptive genotypes for winter season. |

Annex 7. Summary of trial for testing response to seed priming and de-topping

| Particulars | Trial details |
|--|--|
| Number of accessions to be tested | 2 accessions (LRGR 103 and LRGR 111) |
| Number of treatments | <ol style="list-style-type: none"> 1. Variety 1 + Dry seed + No de-topping 2. Variety 1 + Dry seed + De-topping 3. Variety 1 + Primed seed + No de-topping 4. Variety 1 + Primed seed + De-topping 5. Variety 2 + Dry seed + No de-topping 6. Variety 2 + Dry seed + De-topping 7. Variety 2 + Primed seed + No de-topping 8. Variety 2 + Primed seed + De-topping |
| Experimental sites | Chandradaangi-7, Shivagunj, Jhapa district |
| Date of planting | 1 st week of November, 2009 (tentative) |
| Trial design | RCBD with 3 replications |
| Plot size (6 rows) | 5.6 m ² (2.8 x 2) per plot |
| Spacing | 40 x 20 cm (7 rows of 2 m length) |
| Target cropping pattern | Rice fallows in eastern Terai of Nepal |
| Land preparation, manures and fertilizers | As per farmers practice. (Record the quantity of FYM or compost used). Do not apply chemical fertilizers. |
| Plant Population per plot | 70 plants/plot |
| Seed rate | Two seeds per hill but retain only single plant after full establishment |
| Intercultural operations | Two weeding and earthing up No need for any other intercultural operations No stakes provided |
| Observation to be taken | Days to Flowering Days to maturity No of pods/plant Seeds/pod Pod length Total biomass yield Grain yield |
| Experimental data collection, computation and analysis | The observed data will be compiled and analysis will be done to compare the response of the ricebean accessions to de-topping and priming. |

Annex 8: Physiological study on hardseededness in ricebean landraces (2009)

(A Protocol)

Introduction

Hardseededness is a water impermeability related trait particularly prevalent in almost all legume species. Mature and fresh harvested seeds of legume do not germinate readily under favourable environmental condition because of these hard seeds. These seeds may be impermeable to water or gases or seed coat that mechanically be constraint to embryo. It is normally a problem in establishment of plants in field. Besides it also could be a problem in cooking and spoil the preparation of dishes. The hardseededness in legume has ecological importance as in wild species it lengthens the lifespan of viable seeds and in due course of time it overcomes and establish itself. In a preliminary study of hardseededness In ricebean germplasm collected from different districts of Nepal, 10 to 24 % of hardseededness is observed (Table 1). These germplasm represented the landraces from different agro-environments from high-hills to plain area in terai and they vary in seed colour and seed size. The seed coat colour and production environment could have association with hardseededness of seeds in ricebean.

Table 1: Hardseededness in ricebean germplasm under core collection produced at Khumaltar, 2007

| Accessions | District | Seed colour | % hard seeds |
|------------|-------------|-------------|--------------|
| NPGR 05364 | Bhojpur | Yellow | 1.0 |
| NPGR 09391 | Syangja | Mottled | 1.0 |
| LRGR 102 | Palpa | Yellow | 3.0 |
| NPGR 05432 | Baitadi | Yellow | 1.0 |
| NPGR 00007 | Nuwakot | Yellow | 2.0 |
| NPGR 05384 | Mugu | Yellow | 1.0 |
| LRGR 107 | Palpa | Mottled | 1.0 |
| NPGR 08382 | Baglung | Mottled | 3.0 |
| NPGR 00194 | Kabhre | Yellow | 2.2 |
| NPGR 05391 | Bajura | Yellow | 10.0 |
| NPGR 00090 | Dang | Mottled | 1.0 |
| LRGR 129 | Palpa | Yellow | 2.0 |
| NPGR 00073 | Gulmi | Mottled | 1.0 |
| NPGR 05565 | Okhaldhunga | Red | 12.0 |
| LRGR 111 | Gulmi | Red | 1.0 |
| LRGR 42 | Surkhet | Mottled | 6.0 |
| LRGR 99 | Palpa | Yellow | 2.0 |
| NPGR 05373 | Gorkha | Yellow | 24.0 |
| NPGR 00015 | Bhaktapur | Yellow | 10.0 |
| NPGR 06756 | Humla | Yellow | 6.0 |
| NPGR 05382 | Tanahun | Yellow | 6.0 |
| NPGR 00087 | Pyuthan | Yellow | 4.0 |

In a preliminary study of hard-seededness in 50 accessions from 35 districts under the mother trial produced in Khumaltar, 22 accessions showed the occurrence of hard seeds. Most of these were from high-hill districts. However a genotype from Dang, a *terai* district also showed hard seedness.

The methodology of this study was simple by soaking seeds overnight in water, counted down the swelled seeds as sprouts and those not absorbing seeds were wrapped in moist germination paper and counted for sprouting after a week. The seeds remained unsoaked and not sprouted were counted as hard seeds.

Objective:

- to understand the hardseededness of genotypes for field establishment

Expected output:

- Genotypes will be screened for hardseededness, with significance in fields and in kitchen

Methodology

| Particulars | Trial details |
|--|---|
| Number of genotypes | 21 accessions from observation nursery (OBN) |
| Genotypes | NPGR 00005, NPGR 00184, NPGR 00191, NPGR 05422, NPGR 05424, NPGR 05383, NPGR 05381, NPGR 05425, NPGR 05367, NPGR 08381, NPGR 05374, NPGR 05372, NPGR 00189, LRGR 143, LRGR 153, NPGR 05410, LRGR 87, LRGR 113, LRGR 123, LRGR 139 and LRGR 133 |
| Number of trial and experiment site | 1 experiment in seed science and technology research laboratory, ABD, Khumaltar: |
| Fresh harvested seeds used in study | Seeds of OBNs from Rampur, Kabre, Khumaltar (on-stations) and Gulmi (on-farm) will be procured and used for study |
| Trial design | RCBD with 4 replications on 100 seeds in total (25 seeds/rep) |
| Methods/steps | <ul style="list-style-type: none"> • mixing and dividing of sample for preparing the homogenous sample • 100 seeds per sample counted and plated into 4 petri dishes contained equal volume of water (25 seeds each) • Placement of dishes randomly and left in room temperature for overnight • All 25 seeds (swelled and hard seeds) of each rep of each sample plated in moist germination paper and rolled and wrapped upright in a polyethylene bag and left in germinator at 25o C or in room temperature • Counted down the sprouts and hard seeds after a week |
| Observation to be taken | <ul style="list-style-type: none"> • No. of sprouted seeds • No of hard seeds • No. of dead seeds |
| Experimental data collection, computation and analysis | The percent of hard seeds per sample will be calculated and compare the observed data among the samples and analyse for relationships with production environments and seed colour |

Annex 9: Population genetics and out-crossing behaviour study in ricebean (2009)

(A Protocol)

Introduction

The ricebean germplasm characterization has shown to possess many genotypes (varieties), varying in plant types and branching. They also vary in seed coat colour, size and time taken for maturity. During the field evaluation of ricebean genotypes in successive seasons in 2007 and 2008, there observed the harvest of seeds with mixed seed coat colours for most genotypes under study. An understanding of genetics of seed coat colour is important to know the breeding process in ricebean that support improving of ricebean genotypes as a cultigen. Singh and Singh (2004) on segregation pattern of seed coat colour in F₂ and F₃ generations revealed the inheritance of seed coat colour in lentil under control of single dominant gene. Prof John Witcombe therefore suggested a population genetic study to know out-crossing in ricebean. So for the study, he suggested to undertake field studies on dominant colour seeded genotype and recessively light or cream colour seeded genotype in isolation and mixed cultivation surrounded one by other. For this study LRGR 117 (dominant - red colour seeded genotype) and LRGR 91 (recessive – cream colour seeded genotypes) will be used.

Objectives:

- to generate information on genetics of seed coat colour inheritance in ricebean genotypes
- to understand out-crossing in ricebean
- to develop information useful in breeding process in ricebean

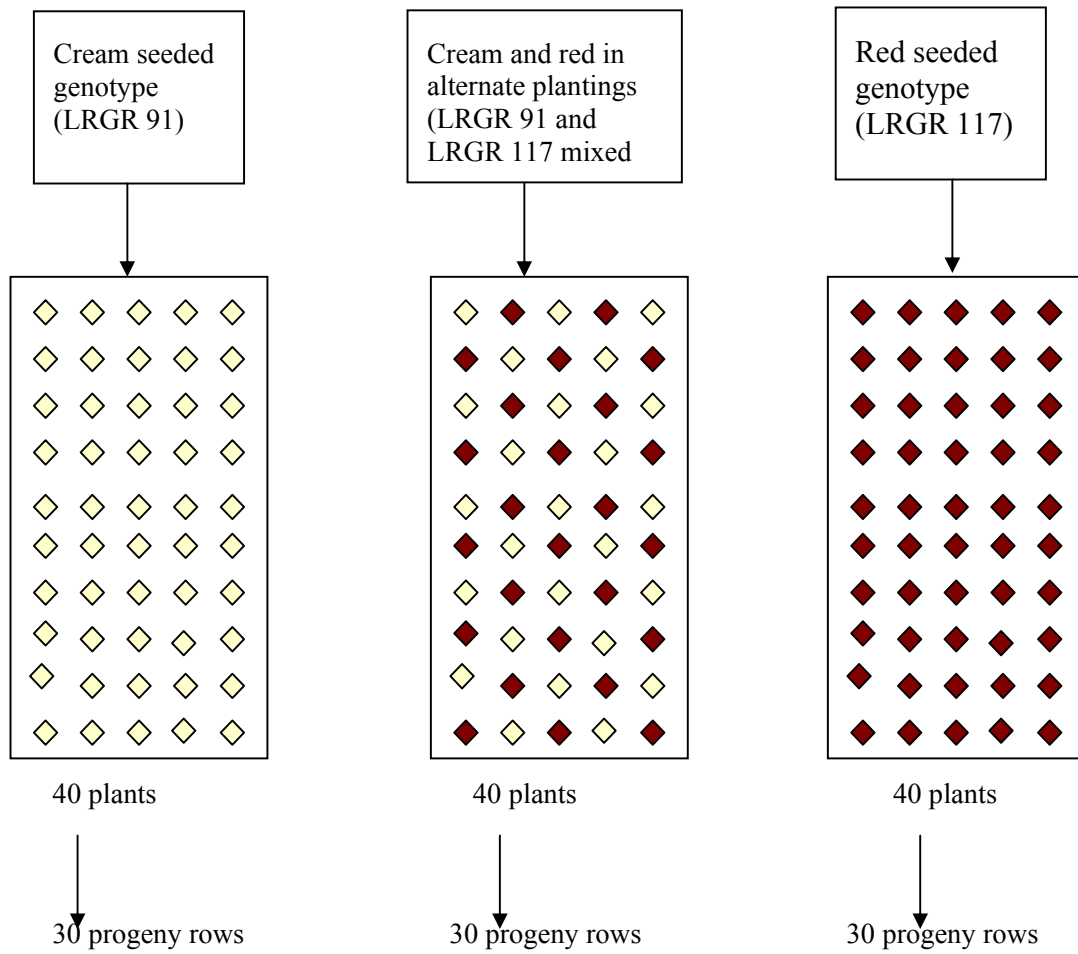
Expected outputs:

- Inheritance of seed coat colour in ricebean and cause of mixed seed coloured harvest will be known.
- Understanding and review on out-crossing in ricebean will be verified
- Useful information in support of breeding in ricebean will be developed

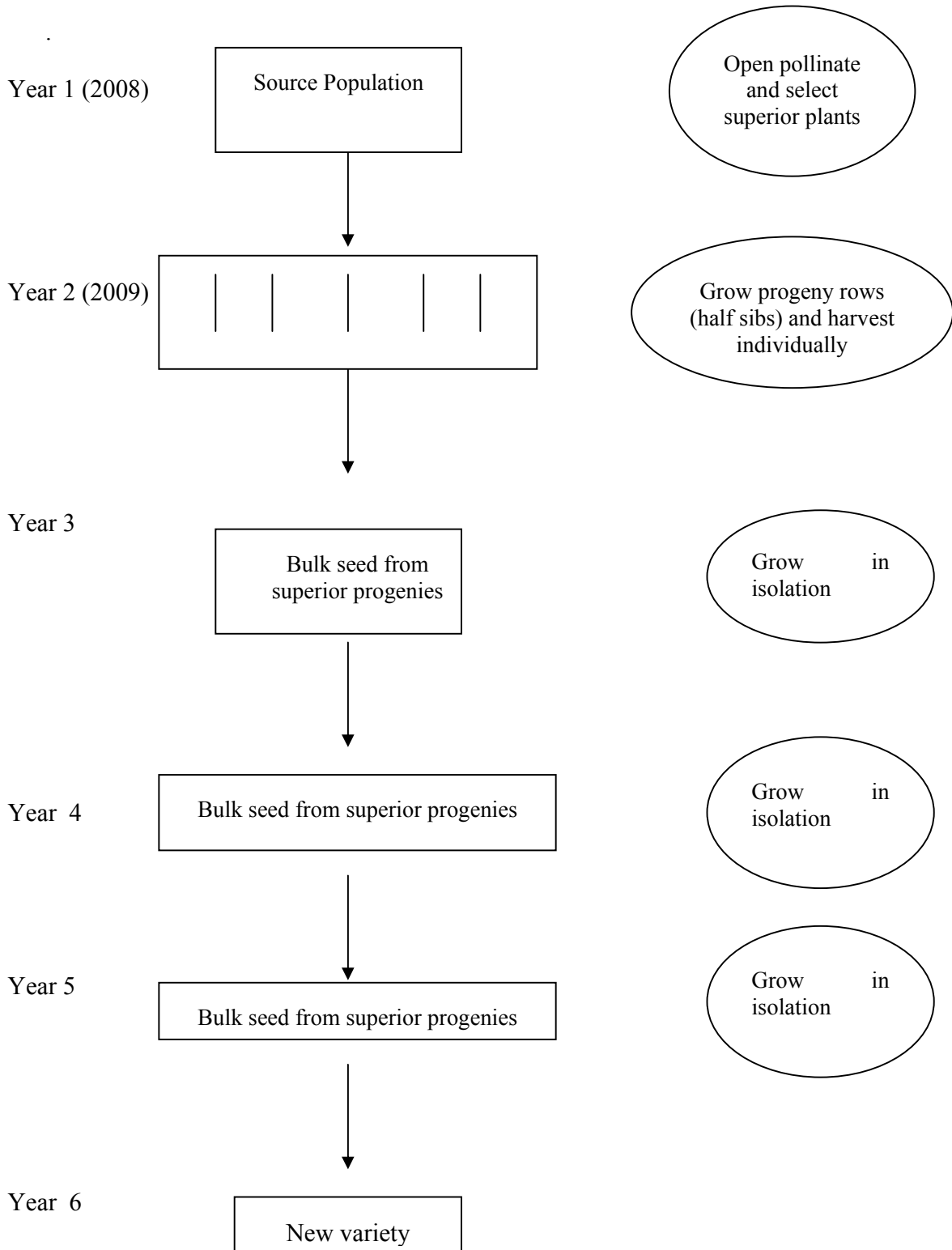
Methodology:

| Particulars | Details |
|--------------------------|---|
| Number of accessions | 2 genotypes: LRGR 91 (bold and cream colour seeded) and LRGR 117 (bold and red colour seeded) |
| Locations | NGLRP, Rampur, Chitwan |
| Trial design | <ul style="list-style-type: none"> • Sole cropping • Isolation plots for each genotypes • Mixed planting of genotypes in alternates in the way to be surrounded by another |
| Planting | In normal season, the seeds will be planted |
| Spacing | 50 x 20 cm |
| Plot size | 40 plants per plot per genotype |
| Intercultural operations | Weeding and earthing up as and when required No chemical fertilizer will be applied but suggested to use FYM Thinning of branches will be carried to avoid intermixing with plants within the plot Other cultural practices and plant protection activities performed as and when required |

Field Layout:



Figurative representation of the population breeding in ricebean



Annex 10. Summary of yield comparison trial for ricebean and blackgram

| Particulars | Trial details |
|--|--|
| Number of accessions of ricebean | 4 accessions from mother trial <ul style="list-style-type: none"> • LRGR 91 • LRGR 111 • NPGR 00008 • NPGR 05364 |
| Number of accessions lines of black-gram | <ul style="list-style-type: none"> • BGL 0069-1 • BGL 003-2-1 • Local |
| Experiment site | Devasthan-3, Dulegaunda VDC, Tanahun |
| Date of planting | 21 August, 2009 |
| Trial design | RCBD with 3 replications |
| Plot size | 6 m ² (3m x 2m) per plot |
| Target cropping pattern | Ghaiya (upland rice or maize based cropping system /option for black-gram |
| Land preparation, manures and fertilizers | As per farmers practice. (Record the quantity of FYM or compost used). Do not apply chemical fertilizers |
| Seed sowing method | Broadcasting |
| Seed rate | 120 seeds/plot |
| Plant Population per plot | 60 plants/plot |
| Intercultural operations | Weeding (2 will be optimum) and earthing up as and when required No need for any other intercultural operations |
| Observation to be taken | Flowering and maturity Total biomass yield and Grain yield |
| Harvesting, threshing | As per the farmers practice. Avoid any chance of varietal mixtures during harvesting and threshing |
| Experimental data collection, computation and analysis | The observed data will be compiled and analysis will be done The results will be used in comparing the yield potential of ricebean and blackgram |