

Characterisation of 4274 accessions of common bean (*Phaseolus vulgaris* L.) germplasm conserved in the Indian gene bank for phenological, morphological and agricultural traits

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Abstract Common bean is one of the most important legume crops worldwide. Response to selection and success of hybridisation in common bean primarily depends on the nature and magnitude of genetic diversity present in the germplasm used. Germplasm comprising 4274 accessions originating from 58 countries were characterized for 22 phenotypic traits for two years. Genetic diversity for traits such as leaf length (4.5–20.7 cm), leaf width (3.4–17.5 cm), pod length (PL) (3.5–23.5 cm), no of pods/plant (4.2–59.6), seeds/pod (2.1–9.6) and 100-seed weight (SWT) (3.5–96.3 g) was observed in the accessions. Based on multivariate analysis, the entire collection was grouped into 10 genetically diverse clusters irrespective of the origin or place of collection of

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Department of Plant Pathology, Himachal Pradesh Agriculture University, Palampur 176 062, Himachal Pradesh, India accessions. First three components obtained through principal component analysis explained 80.44 % of the total variance and it was contributed mainly by PL, pod width (PW), seed length (SL), seed width (SW), pods/plant and SWT. Correlation coefficient of seed weight was positively significant with leaf length, PL, PW, SL and SW while it was negatively correlated with days to flowering, pods/plant and seeds/pod. Regression analysis showed highest direct effect of SW on seed weight followed by SL, and PL. For bean anthracnose, >600 accessions showed resistance under field conditions, however when subjected to screening under artificial conditions against four most prevalent races (03, 515, 598 and 529) of Colletotrichum lindemutianum, we identified 16 accessions which have complete resistance and good agronomic superiority. These accessions may serve as useful genetic material to plant breeders for breeding bean varieties for anthracnose resistance and high yield.

Keywords Characterisation · Common bean · Genetic diversity · Germplasm

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most widely cultivated and consumed legume of the genus *Phaseolus* throughout the world (FAO 2012). It is a

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non-centric crop (Harlan 1971) having two major ecogeographical gene pools-Mesoamerica and the Andes with multiple domestications (Kwak and Gepts 2009). Characterization of both gene pools based on morphology (Singh et al. 1991b; Madakbas and Ergin 2011; Meza et al. 2013; Stoilova et al. 2013), protein types (Gepts et al. 1986), allozymes (Koenig and Gepts 1989), and molecular markers (Rossi et al. 2009; Kwak and Gepts 2009; Khaidizar et al. 2012; La Fuente et al. 2013) has given an overall indication of occurrence of at least two independent domestication events in the two different hemispheres (Debouck and Tohme 1989; Bitocchi et al. 2012). Two large gene pools that pre-existed domestications have large genetic variability in the wild and cultivated common beans (Rosales-Serna et al. 2005; Chacón et al. 2005; Bitocchi et al. 2013).

Little is known about the dissemination of common bean in India especially in the Himalayan region where major genetic diversity exists (Joshi and Rana 1995; Sofi et al. 2014). However, there are strong indications that many Portuguese, English, Dutch, French who travelled to the Indian subcontinent for trading in early part of the 16th century via Red and Arabian Sea; and Chinese through Hindustan Silk Route must have brought nicely colored and easily transportable beans with them (Simmonds 1976; Joshi and Thomas 1987; Joshi and Mehra 1993; Anononymus 2014). The genetic diversity present in India resembles European and Chinese beans, which depicts the combination of both Mesoamerican and Andean cultivated gene pools (Gepts et al. 1986, Debouck and Smartt 1995; Beebe et al. 2001; Logozzo et al. 2007; Angioi et al. 2010; Akbulut et al. 2013). Wide geographic diffusion among different environments and divergent selection criteria for agronomic and organoleptic qualities adopted by the farmers have further enhanced the range of genetic diversity not only in India but different parts of the world (Rana et al. 2010; Kumar et al. 2009; Singh 1989; Santalla et al. 2002; Meza et al. 2013). Presently, principal cultivated beans in India are dark and light red kidney, white kidney and cranberry beans with seed weight >40 g from Andean gene pool and medium and smallseeded (<40 g) with wide range of colours including white, cream, pinto, navy, yellow, black from Mesoamerican gene pool (Fig. 1).

In India, dry beans are grown in 9,100,000 ha with production of 3,630,000 tons and yield 399.0 kg/ha as

compared to World's area 29,234,228 ha with production 23,139,004 tons and yield 792 kg/ha (FAOSTAT 2013). Here, it is popularly called as *rajmash* and mainly produced under rainfed conditions in the traditional production system that include rotation with vegetables and intercropping of climbing beans with grain amaranth and maize during rainy season in the mountains while as sole crop of bush types during winters in the Indian plains (Santalla et al. 2004; Sharma and Rana 2005; Tiwari et al. 2005; Sharma et al. 2006; Rana et al. 2012). Common bean is an important source of minerals, i.e., iron and zinc, and many vitamins (Limongelli et al. 1996; Kaur et al. 2009; Singh Narpinder et al. 2010; Khetan et al. 2015), therefore, considered an economically, nutritionally, and socially important crop (Broughton et al. 2003; Pujolá et al. 2007; Mishra et al. 2010; Singh et al. 2012; Parmar et al 2014). In local gastronomy, it is associated with several cultural and culinary traditions (most famous kidney bean and rice based recipe in India is called rajmah-chawal), usually based on the use of local seed production of landraces worldwide (Masi et al. 1999; Polegri and Negri 2010; Negri 2012, Rana et al. 2012; Mercati et al. 2013). The landraces grown in the high mountain regions are highly appreciated and fetch up 2-3 times higher market price than those grown in the plains (Paudel et al. 2003; Rai et al. 2010, Rana et al. 2011; Bist and Rana 2012; Bisht and Rana 2012). Most famous landraces grown in the north-west part of India are known as 'Chamba rajmah' 'Barot' 'Kinnauree' 'Auli' 'Munsiyari' 'Harshil' 'Bhaderwah' and 'Kashmiri'. These landraces have been named on the basis of geographical region in which they are grown. High priority has been accorded to protect these landraces, which are differentiated by morphological, agronomic and nutritional traits and highly adapted to local microclimatic conditions of their limited geographical areas. Largely, farmers in the traditional production systems grow as many as 10–12 landraces together (Fig. 2), but economic considerations and agronomic developments have led to changed emphasis on single-component varieties over multi component mixtures. As a result, displacement of traditional genetic diversity is now visible not only in the high cropping intensive areas but in the traditional kidney bean growing areas also (Rana et al. 2000; Sofi et al. 2014).

The genetic diversity, which has been collected from different parts of India and introduced from abroad, has been conserved in the national gene bank



Fig. 1 Genetic diversity in seed, shape and color in the kidney bean germplasm

under long term storage at New Delhi and medium term storage (MTS) at Shimla. However, the value of germplasm conserved in the germplasm bank depends on the information generated through its characterisation and evaluation for different traits (Gepts 2006; Gonçalves et al. 2009; Blair et al. 2010). In this study,



Fig. 2 Farmers grow many landraces together and also as sole crop in the mountain region of India

we undertook large-scale phenotypic characterization of full range of genetic diversity to select genetically diverse accessions from 4274 accessions conserved in the MTS. Several authors have emphasised that phenotypic and phenological information based on descriptors continues to be the first step for the assessment, description and classification of large germplasm collections to enhance their use in research (Tar'an et al. 2005; Bhullar et al. 2009; Freitas et al. 2010; Szilagyi et al. 2011; Raggi et al. 2013). This paper will give plant breeders an insight of the genetic diversity present in the common bean germplasm in India, which may facilitate their indents to gene bank for germplasm of their own choice.

Materials and methods

Plant material

The plant material used in this study comprised 4274 accessions, of which 2308 were indigenous collections collected from 18 different states primarily from the Indian Himalaya (1887 accession) and 1966 were exotic collections representing 57 countries of the world (Table 1). Majority of EC accessions represent the

countries from Mesoamerican and Andean centres of domestication including Mexico, Colombia and Peru. Data on the germplasm were recorded during two vegetative cycles of 2011 and 2012 in the experimental fields at Regional Station of National Bureau of Plant Genetic Resources (NBPGR 2000) Shimla, Himachal Pradesh. This farm is located at 31°05′53.89″N and 77°09'34.92"E and elevation of 1924 m above sea level. The soil of the experimental farm is sandy loam inclined more towards skeletal nature, dry, shallow and moderately rich in organic matter. Average annual rainfall is 1250 mm, of which around 60-65 % is received during the months from July to September. Mean maximum and minimum temperature ranges between 33.3 and -3.1 °C. The crop was sown in the month of June in each year in 2 rows of 2 m length with 30 cm inter-row spacing for determinate type and 75 cm for indeterminate types and harvested in October-November depending upon the maturity period of accessions. We followed standard cultivation practices recommended for growing common bean in the Indian hill region.

Plant characters and data recording

The data were recorded on 22 traits, of which 11 traits viz. days to flowering (DF), leaflet length (LL), leaflet

Table 1Country oforigin/source for 4274accessions used in the study

| Country | Accs. | Country | Accs. | Country | Accs. | |
|----------------|-------|-------------|-------|--------------------------|-------|--|
| Afghanistan | 2 | Georgia | 6 | Nicaragua | 7 | |
| Argentina | 10 | Germany | 25 | Peru | 213 | |
| Australia | 9 | Guatemala | 61 | Portugal | 5 | |
| Belgium | 7 | Haiti | 12 | Romania | 1 | |
| Belize | 1 | Holland | 8 | Russia | 10 | |
| Bolivia | 16 | Honduras | 7 | Rwanda | 14 | |
| Brazil | 60 | Hungary | 33 | Slovakia | 23 | |
| Bulgaria | 22 | India | 2316 | Spain | 12 | |
| Burundi | 11 | Iran | 3 | Sweden | 2 | |
| Cameroon | 6 | ISRAEL | 12 | Tanzania | 5 | |
| Canada | 3 | Italy | 2 | Turkey | 12 | |
| Chile | 9 | Jamaica | 1 | United Kingdom | 12 | |
| Colombia | 582 | Japan | 3 | Uganda | 3 | |
| Congo | 9 | Kenya | 6 | Ukraine | 3 | |
| Costa Rica | 11 | Liberia | 1 | United States of America | 285 | |
| Czechoslovakia | 5 | Macedonia | 2 | Ussr | 29 | |
| Ecuador | 45 | Malawi | 14 | Venezuela | 2 | |
| El Salvador | 7 | Mexico | 269 | Zambia | 17 | |
| Ethiopia | 1 | Nepal | 18 | | | |
| France | 2 | Netherlands | 2 | | | |

width (LW), pod length (PL), pod width (PW), pods per plant (PPP), days to maturity (DM), seed length (SL), seed width (SW), seeds per pod (SPP), 100-seed weight (SWT) were measured quantitatively, while another 11 traits viz. early plant vigour, plant growth habit, stem pigmentation, flower colour, leaflet shape, pod colour, pod pubescence, pod shape, seed colour, seed mottling, and bean anthracnose score were assessed qualitatively on different scales. All the traits were measured/assessed based on *P. vulgaris* descriptors published by IBPGR (1982) on randomly selected five plants of each accession.

For further screening of field resistant lines under artificial conditions against anthracnose, germinated seed dip method of inoculation as described by Champion et al. (1973) was used for evaluation of resistance. Three seeds of each accession were surface sterilized with 0.1 % mercuric chloride followed by three washing with sterilized water were placed on double layers of seed germination paper covered by butter paper to retain the moisture and then kept at 25 ± 1 °C for germination in incubator. After 3 days, seed coats of germinated seeds were removed and dipped in standard spore suspension for 2–3 min. Thereafter seeds were sown in 3 cm deep plastic trays containing sterilized river sand. The trays were kept in growth chamber at 22 + 1 °C with >90 % humidity and 12 h photoperiod for 6 days. The disease reaction was recorded by using 0–5 point scale as described by Drijfhout and Davis (1989), where (0) no disease symptoms, (1) pinpoint lesions, (2) small lesions, (3) large sunken lesions, (4) large deep sunken lesions, (5) graded as susceptible. Plants showing 0, 1 and 2 or either of these were recorded as resistant (r) while those showing 3, 4 and 5 or either of these graded as susceptible (s). Each accession that showed resistance was tested twice to confirm the reaction.

Statistical analysis

We evaluated the germplasm in Augmented Block Design (Federer 1956) with six standard check varieties viz. Jawala, Baspa, Triloki, Kailash, Kentucky Wonder, and Laxmi in 12 blocks. The means were adjusted using online software for augmented block design developed by Rathore et al. 2004. Before undertaking statistical analysis on the basis of adjusted pooled mean values, homogeneity of variance was tested as per Levene (1960). Frequency distribution graphs were obtained for all the traits. The quantitative traits were further analysed for various statistical parameters viz. mean, range, variances, correlations, genetic diversity to find out genetic similarity/dissimilarity and principal component analysis (PCA) using the statistical software SYSTAT-12. Phenotypic and genotypic coefficients of variation (PCV and GCV) for each trait were computed as PCV = $\sqrt{V_P}$ mean \times 100, GCV = $\sqrt{V_G}$ /mean \times 100 as per (Burton 1952) and categorized the range as per Sivasubramanian and Madhavamenon (1978). Broad sense heritability was estimated as h^2 (bs) = V_G/V_P \times 100 as per (Lush 1940) and further classified into low, medium and high (Robinson 1996). Expected as EGA = k × V_G/V_P × $\sqrt{V_P}$ as per Johnson et al. 1955. Here the standard value of k is 2.06 assumed at 5 % selection intensity; V_G is genotypic variance; and V_P is phenotypic variance. Genetic advance was expressed as % of mean as GA (%) = EGA/mean \times 100. The significance of variances was tested at the 5 % probability level.

Results

Frequency distribution for qualitative traits

All the traits measured qualitatively showed wide range of variation among 4274 accessions evaluated (Fig. 3). Here in the text we have discussed only those traits and per cent accessions for which frequency of their occurrence was high while detail of all frequencies have been described in Figs. 3, 4. Early plant vigour, which determines the subsequent growth of plant, was good to medium for 98 % accessions. Indeterminate growth habit was observed for 43 % accessions, determinate for 28 and 29 % were intermediate type. The shape of leaflet was ovate for 83 % accessions, round for 14 % and ovate-lanceolate for others. The flower colour was white (39 %) followed by pink (26 %) and lilac (21 %). Green pod colour was predominant with different intensities of dull to shiny green for 87 % accessions. The surface of pods was glabrous for 96 % accessions while pod shape was straight for 69 % accessions. Wide range of seed colours such as white, red, maroon, pink, purple, yellow, black, brown along with varying tonalities was obtained, however, white (31 %) and red (29 %) were predominant. Around 66 % accessions have single seed coat colour while 34 % had mottled seed coats of various colours. We also undertook field screening of accessions against four most prevalent races viz. 03, 515, 529 and 598 of bean anthracnose (*Colletotrichum lindemuthianum*) causing serious crop loss to bean crop in the Himalayan region (Sharma et al. 2012). Out of 4274 accessions, 1287 were categorised as highly susceptible (scored 4) and, 1618 susceptible (3), 683 moderately resistant (2), 686 resistant (1) and 228 (0) as highly resistant to the disease anthracnose.

Frequency distributions graphs for quantitative traits depicted in the Fig. 4 showed that >70 % accessions fall in the medium group of flowering (46–65 days) and maturity periods (90–130 days). LL and LW revealed wide variation ranging from 4.5–20.7 to 3.4–17.5 cm, respectively. Around 80 % accessions had medium PL (10–16 cm) and PW (0.9–1.2 cm). High variability was recorded for number of pods/plant and it ranged from 10 to25 for 67 % accessions. The SL was 0.5–1.5 cm for 75 % accessions while SW was 0.6–1.0 cm for 74 % accessions. The most common numbers of seeds per pod were 4–8 and occurred in 83 % accessions. The seed weight was found highly variable trait and varied from 3.5 to 96.3 g/100 seed weight.

Mean, range, variance, coefficient of variance, heritability and genetic advance

Homogeneity of variance was tested using the Levene's test showed p value 0.93, which was higher than the chosen alpha level (0.05) indicating that the variances of both the years were homogeneous allowing us to do subsequent statistical analysis. The statistical analysis of data on quantitative traits showed wide range of variability among the accessions (Table 2). The mean numbers of days to 50 % flowering were 55, but it ranged from 27 for accession IC370764 to 104 for EC500268 and EC500396. LL and width were highest for EC500242 and EC024955 with an average of 11.5 cm and 8.6 cm, respectively. PL was longest (22.5 cm) for IC328871 and EC271552 and shortest (3.4 cm) for EC531041 with an average value of 12.5 cm. EC500431 has widest pod (2 cm) while it was narrowest (0.4 cm) for IC321182. The average number of pods/plant was 16.3 while range was 4.2-49.6 for IC556535 and EC500299, respectively. PI301808 took longest time of 167 days for maturity as compared EC0944456, which matures in 71 days. IC383388 had maximum



Fig. 3 Frequency distribution of accessions for qualitative traits

SL (2.03 cm) and width (1.2 cm) while IC383008 has maximum number of seeds/pod (10) compared to IC417359, which has minimum (2). The average SWT

for all accessions was 29.5 g but it was highest for IC417355 (102.3 g) compared to HUR69 (3.6 g). PCV and GCV were high for pods/plant, SWT, SW,



Fig. 4 Frequency distribution of accessions for quantitative traits

PW, SL and LW and low for rest of the traits. The heritability was found high for all the traits and it was ranging from 71.3 % for DM to 91.3 % for PL. The

genetic advance expressed as % of mean was low (<35) for DM, DF, PL, and SPP and high (>35) for rest of the traits.

 Table 2
 Statistical parameters of genetic variability in 4274 accessions of common bean

| Traits | Range | Mean + SE | Variance (P) | Variance (G) | PCV (%) | GCV (%) | Heritability | Genetic advance as % of mean |
|--------|------------|----------------|-----------------|-----------------|------------|------------|--------------|---------------------------------|
| DF | 27.0-104.0 | 55 ± 2.6 | 87.7 | 68.3 | 17.0 | 15.0 | 77.8 | 27.0 |
| LL | 4.5-20.7 | 11.5 ± 1.1 | 4.8 | 4.2 | 19.1 | 17.8 | 87.5 | 36.1 |
| LW | 3.4-17.5 | 8.6 ± 1.3 | 3.4 | 3.1 | 21.4 | 20.4 | 91.1 | 40.2 |
| PL | 3.5-23.5 | 12.5 ± 1.7 | 4.6 | 4.2 | 17.2 | 16.3 | 91.3 | 32.1 |
| PW | 0.4–2.0 | 1.2 ± 0.06 | 0.1 | 0.08 | 26.3 | 23.5 | 80.0 | 43.4 |
| PPP | 4.2-59.6 | 16.3 ± 1.3 | 38.2 | 30.6 | 46.5 | 41.5 | 80.1 | 76.5 |
| DM | 71.0-166.0 | 104.2 ± 4.6 | 170.1 | 121.3 | 12.5 | 10.5 | 71.3 | 18.3 |
| SL | 0.5-2.03 | 1.2 ± 0.01 | 0.07 | 0.06 | 22.0 | 20.4 | 85.7 | 38.6 |
| SW | 0.2-1.2 | 0.65 ± 0.01 | 0.05 | 0.04 | 34.4 | 30.7 | 80.0 | 56.7 |
| SPP | 2.0-10 | 5.1 ± 0.9 | 0.79 | 0.65 | 17.4 | 15.8 | 82.2 | 29.4 |
| SWT | 3.5-96.3 | 29.5 ± 2.2 | 119.0 | 102.0 | 36.9 | 34.2 | 85.7 | 67.7 |

DF Days to flowering, *LL* leaflet length, *LW* leaflet width, *PL* pod length, *PW* pod width, *PPP* pods per plant, *DM* days to maturity, *SL* seed length, *SW* seed width, *SPP* seeds per pod, *SWT* 100-seed weight

 Table 3 Correlation coefficients of different quantitative traits

| Traits | DF | LL | LW | PL | PW | PPP | DM | SL | SW | SPP |
|--------|--------|-------|-------|--------|-------|--------|-------|--------|-------|--------|
| LL | -0.04 | | | | | | | | | |
| LW | 0.01 | 0.60* | | | | | | | | |
| PL | -0.14 | 0.25* | 0.25* | | | | | | | |
| PW | -0.06 | 0.14* | 0.14* | 0.34* | | | | | | |
| PPP | 0.10* | 0.15* | 0.23* | -0.01 | -0.04 | | | | | |
| DM | 0.57* | 0.02 | 0.08 | -0.14* | -0.07 | 0.10* | | | | |
| SL | -0.09* | 0.15* | 0.09 | 0.46* | 0.32* | -0.11* | -0.09 | | | |
| SW | -0.03 | 0.11* | 0.04 | 0.19* | 0.24* | -0.10* | 0.02 | 0.45* | | |
| SPP | 0.15* | 0.07 | 0.14* | 0.02 | -0.07 | 0.28* | 0.16* | -0.11* | -0.06 | |
| SWT | -0.10* | 0.14* | 0.07 | 0.34* | 0.30* | -0.21* | -0.02 | 0.49* | 0.34* | -0.13* |

* Significant at P = 0.05

DF Days to flowering, LL leaflet length, LW leaflet width, PL pod length, PW pod width, PPP pods per plant, DM days to maturity, SL seed length, SW seed width, SPP seeds per pod, SWT 100-seed weight

Correlation and regression

The matrix developed for correlation coefficients (Table 3) showed significant positive correlation of seed weight with LL, PL, PW, SL and SW and negative correlation with DF, seeds/pod and pods/-plant. Seeds/pod had positive correlation with DF, leaf width, pods/plant and DM while negative correlation with SL. Other combinations of traits, which have strong positive correlations among themselves were leaf length & leaf width, DF & DM, PL & PW, PL &

SL, PW & SW and SL & SW. Least square regression analysis performed for seed weight versus other traits showed that SW had highest direct effect on seed weight followed by PW, SL, PL and leaf length while seeds/pod, pods/plant and leaf width had negative direct effect. The plot of residual versus fitted values showed that residuals of majority of the accessions bounce randomly around 0 line forming horizontal band (data not shown). The variances of the error terms were equal, relationship among accessions was linear and there were only 74 outlier accessions out of 4274.

| Cluster variables | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 |
|-------------------|------|-------|-------|------|-------|-------|------|------|-------|-------|
| DF | 55.5 | 55.6 | 71.8 | 44.9 | 52.8 | 62.5 | 46.6 | 52.1 | 65.3 | 54.4 |
| LL | 10.8 | 12.1 | 11.1 | 11.1 | 11.4 | 11.7 | 11.8 | 12.0 | 11.2 | 11.8 |
| LW | 8.1 | 8.5 | 8.5 | 8.2 | 8.6 | 9.4 | 8.6 | 9.3 | 8.5 | 8.7 |
| PL | 11.7 | 13.8 | 11.8 | 13.0 | 11.7 | 12.8 | 13.8 | 12.0 | 11.8 | 12.8 |
| PW | 1.2 | 1.3 | 1.1 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| PPP | 10.5 | 10.9 | 15.3 | 10.4 | 13.6 | 12.9 | 11.1 | 22.9 | 15.6 | 11.5 |
| DM | 97.6 | 101.4 | 128.8 | 88.9 | 116.9 | 123.4 | 91.7 | 96.7 | 110.8 | 106.0 |
| SL | 1.1 | 1.5 | 1.1 | 1.3 | 1.1 | 1.3 | 1.4 | 1.1 | 1.2 | 1.3 |
| SW | 0.6 | 0.7 | 0.6 | 0.6 | 0.5 | 0.6 | 0.6 | 0.5 | 0.5 | 0.6 |
| SPP | 5.1 | 4.9 | 5.4 | 4.9 | 5.3 | 5.2 | 4.9 | 5.4 | 5.3 | 5.4 |
| SWT | 20.6 | 51.7 | 24.7 | 26.5 | 22.6 | 42.1 | 41.0 | 21.5 | 23.2 | 36.2 |
| No. of accessions | 405 | 436 | 555 | 537 | 424 | 517 | 224 | 562 | 346 | 268 |

Table 4 Mean values of different variables in 10 clusters and no. of accessions in each cluster

DF Days to flowering, *LL* leaflet length, *LW* leaflet width, *PL* pod length, *PW* pod width, *PPP* pods per plant, *DM* days to maturity, *SL* seed length, *SW* seed width, *SPP* seeds per pod, *SWT* 100-seed weight

Genetic diversity and principal component analysis

Based on the multivariate analysis performed on 11 quantitatively measured traits, all the 4274 accessions were grouped into 10 clusters and each cluster was found to have varied number of accessions (Table 4). However, it was not feasible to produce a dendrogram due to large number of accessions involved in the analysis. The number of accessions falls in each cluster were highest (562) in cluster 8 (C8) followed by C3 (555), C4 (537), C6 (517), C2 (436), C5 (424), C1 (405), C9 (346), C10 (268) and C7 (224). The mean value of accessions grouped into each cluster (Table 4) showed that accessions in C4 were early in flowering and maturity whereas all accessions of C3 had late maturing types. The accessions with high average number of pods/plant (22.9) were grouped into C8 while seed weight was high (51.7) for accessions grouped in C2, followed by C6 (42.1) and C7 (41.0). High mean (>13 cm) PL and SL (>1.4 cm) were observed for accessions grouped into C2, C4 and C7. Genetic distance measured through multivariate analysis between different clusters was highest in C3 & C4 followed by C3 & C7, C3 & C2, C3 & C8, C6 & C8, C3 & C1, C6 & C7, C1 & C8, C5 & C9, C1 & C4, C4 & C7, C2 & C10 and C7 & C10.

The PCA used to eliminate the redundancy in data set revealed that all the 11 quantitatively measured traits have been loaded on first five components,



Fig. 5 Biplot of different variables loaded on PC1 and PC2

however, major portion of variance (80.4 %) in bean germplasm is explained by first three components (Fig. 5). The first component (PC1) accounted for 34.5 % of variation, through PL, PW, SL, SW and SWT; PC2 accounted for 27.5 % of variation loaded on leaf length, leaf width, pods/plant and seeds/pod and PC3 contributed 18.4 % variance through DF and DM. PC4 and PC5 shared 8.8 and 7.3 % variance, respectively, and loaded partially on PW, seeds/pod and SW.

Discussion

Common bean ranks high in agriculture worldwide because of its nutritious food components and good market price (Rodiño et al. 2006; Rana et al. 2010; Singh et al. 2012; Khetan et al. 2015). The analysis of genetic diversity in large collections conserved in the gene banks is important for deciphering nature and magnitude of variability, genetic relationship between traits for the efficient management and use of germplasm (Stoilova et al. 2005; 2013 Blair et al. 2010; Szilagyi et al. 2011). The simplified phenotypic approach has been found useful for preliminary characterisation and discrimination of accessions to understand the level of genetic diversity (Foschiani et al. 2009; Atilla et al. 2010; Szilagyi et al. 2011). It helps in detecting the occurrence of diversity within population and spelt out precise information on the nature and magnitude of genetic divergence among gene pools for reliable scoring during selection of potential parents for hybridisation (Szilagyi et al. 2011; Mercati et al. 2013; La Fuente et al. 2013).

This study describes one of the first large-scale phenotypic characterisation of the bean collection conserved in the Indian gene bank. The germplasm presented wide range of genetic variability for 22 traits among 4274 accessions. The frequency distribution obtained for 22 traits showed the presence of maximum possible range of variability reported in the Andean and Mesoamerican gene pools of common bean (Singh et al. 1991a; Bitocchi et al. 2012; La Fuente et al. 2013). Among different traits, seed traits have been found most important in common bean and major determinants of commercial acceptability of varieties (Park et al. 2000; Bisht et al. 2014; Rana et al. 2014). Seed traits have also been considered highly heritable traits, therefore important in breeding programmes (Singh et al. 2007; Blair et al. 2010). We found marked variation in seed colour ranging from a single colour viz. white, red, maroon, pink, purple, yellow, black, and brown to mottled seeds with varying tonalities. The preference for seed colour was found varied from place to place in India and it was kidney shaped red, maroon, pink, and yellow beans, which find premium place among bean consumer and producer (Sharma and Rana 2005; Rana et al. 2010). Similar observations on seed colour preference have been recorded in other parts of the world (Trutmann et al. 1996; Wortmann et al. 1998; Fonseca et al. 2007). This suggests that seed colour genes have perhaps been moved between gene pool groups through hybridization, inter-gene pool introgression and extensive selection for different preferences by producers and consumers worldwide. Apart from seed colour, variation found in seed shape and size was significantly wider (3.5–96.3 g/SWT) in the accessions. These results are consistent with findings of different scientists who have reported wide variation in seed shape and size in bean germplasm (Rodiño et al. 2003; Rai et al. 2006; Rodiño et al. 2006; Cabral et al. 2010; Lioi et al. 2012). The most elaborated work done by Singh (1989) and Singh et al. (1991a, b) have given high taxonomic value for seed size, colour and shape in comparison to the vegetative characteristics of plant in common bean.

Apart from seed traits, plant growth habit was found varied from bushy to aggressive climbing type. The predominance of one growth habit type is related to ecological adaptation as well as to the cropping system being followed. For instance, in the hill regions beans are generally intercropped with maize and amaranth for which climbing types are more desirable while in the plains beans are grown as sole crop, bush types are preferred. We observed that climbing bean generally have longer life cycle, higher productivity and late maturity while bush type have early maturity, short plant and low productivity. These observations are in agreement with studies conducted by García et al. (1997) who demonstrated that increasing climbing ability increase the time needed for achieving physiological maturity, and plants with indeterminate growth are more productive than plants with determinate growth habit. Piergiovanni and Lucia (2010) described that 90 % of Italian landraces cultivated in Basilicata region have climbing habit. It was found that flowering and maturity were also found to be influenced by environmental parameters viz. temperature and photoperiod length in both bush and climbing types. These results obtained here are in agreement with those obtained in bean germplasm from Iberian Peninsula (Rodiño et al. 2003), Nicaragua (Gómez et al. 2004), Mexico (García et al. 1997), Portugal (Coelho Rita et al. 2009) and Andean cultivars (Santalla et al. 2004).

The extent of genetic variation could also be better judged by the estimation of GCV in relation to PCV. Small difference between the two indicates that observed variation and expression of traits is mainly due to genetic factors while larger difference indicate role of environment. However, these parameters of variability alone may not be sufficient for determining of magnitude of heritable variation. High GCV coupled with high heritability and genetic gain is useful for predicting the result of selection based on phenotypic performance. In our germplasm, LL, LW, PL, PW, PPP, SL, SW and SWT showed high level of variance coupled with high heritability and genetic advance. This suggests that these traits may be under the influence of additive gene interactions and use of simple selection methods may bring significant improvement for these traits. Accessions such as IC328541, IC531148, IC326556, EC25506, HUR116, IC328871, IC199249, EC500431, EC500951, EC500299, IC14913 IC383388, NC67302, IC641138, PI1044643, IC417355, NC56079 and IC340902 showed high values for these traits and can be subjected for further testing and selection for yield gains through simple selection based on these traits. On the other hand flowering, maturity and seeds/ pod, which have low variance, heritability and genetic advance showed the presence of non-additive gene interactions and needed to be improved by hybridization (Johnson and Gepts 2002; Raffi and Nath 2004; Kumar 2008; Ahmad and Kamaluddin 2011; Rai et al. 2010; Sharma et al. 2012). Accessions viz. IC338701, IC340928, IC328541, EC25501, EC398509, IC383008, IC417353 and IC340964 could be used in the hybridisation for improving on traits showing low heritability and genetic advance.

Correlation coefficient is important in plant breeding because it measures the degree of association (genetic and non-genetic) between two or more traits (Dewey and Lu 1959). In the presence of high correlation between two traits, selection for one trait will cause a change in its mean through additive gene effects of selected individuals and simultaneously cause an indirect change in the mean of the other trait (Dursun 2007; Apostolova et al. 2009; Kumar et al. 2009; Singh et al. 2011; Mudasir et al. 2012). The significant positive correlation of seed weight with LL, PL, PW, SL and SW showed that selection for any of these traits may favour improvement in other traits also, while negative correlation with DF, PPP and SPP may adversely affect the gain. The data on the accessions showed that small leaf and pod size invariably have smaller seeds while large seeded accessions had broader leaf and medium to long and wide pod. The positive association of seed weight with various other yield traits have been reported in bean germplasm (Rai et al. 2006; Blair et al. 2006; Dursun 2007; Bhushan et al. 2008; Apostolova et al. 2009; Krasu and OZ 2011; Kumar et al. 2009; Sofi et al.

2011; Ahmed and Kamaluddin 2013; Sofi et al. 2014). According to Blair et al. (2009), varieties of dry beans that do not meet minimum seed weight criteria can be rejected by the market place while in snap beans varieties with elongated seed shape or smaller seed size are preferred as they are correlated with pod quality. Based on the correlation coefficients, it is possible to construct/select a plant type integrating yield components such as LL, PL, seed weight, seed diameter into one, however, to combine few more traits like pods/plant, seed/pod and early maturity, common bean breeder has to go for extensive crossing followed by selection.

Grouping large number of germplasm accessions into few numbers of homogenous clusters facilitates the selection of diverse parents. It permits precise comparison among all the possible pair of populations and provide an opportunity for bringing together gene constellation yielding desirable progenies. The maximum and minimum difference observed between clusters III & IV and clusters I and VIII, respectively indicating genetic distance and closeness among accessions due to different genetic constitutions. Meaning thereby clusters that have higher mean value and genetically distant could facilitate the selection of accessions for use in the breeding programmes (Sharma et al. 2009; Rana et al. 2013; Meza et al. 2013; Stoilova et al. 2013). The varying levels of similarity and dissimilarity in common bean landraces have been observed in various other studies (Piergiovanni et al. 2000; Franklin et al. 2009; Biswas et al. 2010; Szilagyi et al. 2011; La Fuente et al. 2013). The present collection represents 58 countries of the world but while grouping accessions into various clusters we did not observe any favour to a specific geographical origin. The accessions were grouped into various clusters irrespective of the country/region from where these have been collected/procured. This suggests that due to the free exchange of seed materials among different regions and genetic constellations of character might be associated with particular region in nature, but lose their individuality under human interference (Ramanjulu et al. 1999; Singh et al. 2002; Adesoye and Ojobo 2012). Scientists have discussed genetic drift, selection pressure and environment as other major factors that could cause greater diversity than geographical distance (Sicard et al. 2005; Nehvi et al. 2007; Kwak and Gepts 2009; Angioi et al. 2010).

Similarity indices and pattern of relationships obtained through genetic diversity and PCA are useful to evaluate potential breeding value of germplasm through traits loaded on various components (Keneni et al. 2005). Using PCA, we were able to quantify the degree of divergence between populations to understand the trend of their evolutionary pattern and to assess the relative contribution of different components to the total divergence together with nature of forces operating at intra and inter-cluster levels (Sharma et al. 2009). In the present study, 34.55 % of the total variation was contributed by PL and width, SL and width and seed weight while 27.52 % by leaf length and width, pods/plant and seed/pod indicating that seed and pod traits have contributed highest genetic diversity in common bean germplasm. The results are in agreement with various other studies that reported the maximum contribution of seed weight, pods/plant and seed length towards genetic divergence in common bean (Park et al. 2000; Mirjana 2005; Ceolin et al. 2007; Misra et al. 2010).

Besides, understanding the nature and magnitude of genetic diversity obtained through different traits, it is desirable to indentify the accessions found resistance against most prevalent diseases and pests. Here we simultaneously screened the accession for bean anthracnose, which causes yield losses up to 100 % (Fernandez et al. 2000; Miklas et al. 2006; Sharma et al. 2007). The pathogen possess high degree of pathogenic variability throughout the world including India and >100 races of C. lindemuthianum have been identified worldwide (Rodriguez-Guerra et al. 2003; Gonzalez et al. 2004; Mahuku and Riascos 2004; Sharma et al. 2007). Its management can be done by using certified seed, crop rotation, seeds and foliar treatment with fungicides and genetically resistant varieties. Out of these most of strategies are not followed by marginal and resource poor farmers particularly those living in the mountains and grow bean as major pulse crop. Under such situation use of genetically resistant varieties offer most effective, least expensive and easy to adopt way to the farmer. In the present collection, >600 accessions showed resistance to bean anthracnose under field conditions. Out of >200 accessions indentified as highly resistant in the field 99 had been screened against 4 races viz., 03, 515, 529 and 598 of C. lindemuthianum under laboratory conditions by Sharma et al. (2012). Out of 99 accessions 65 showed resistance to one or other race but we selected 16 accessions viz., IC328537, IC328538,

IC448888, IC313194, IC278723, IC339645, IC398530, EC169813, EC398530, EC500226 IC326964, IC37 0764, EC77006, EC50063, EC50079, and EC530926 which were found resistant against all the races and also showed agronomic superiority. We assumed that being resistant and agronomically superior there accessions could be a good breeding material for imparting anthracnose resistance to bean varieties along with high yield gains.

Conclusion

Progress in breeding high yielding varieties of common bean has been modest and one of the constraints regarded by many plant breeders is the narrow genetic base available within the breeders' stock (Cooper et al. 1997; Kelly et al. 1998; Perseguini et al. 2011). Given this constraint, plant breeders need genetically diverse germplasm not only for yield but its contributing traits also. The results obtained based on various genetic parameters analysed on wide range of traits, especially seed and pod traits; it is concluded that determinant genetic effects of the phenotypic expression of these traits are fundamentally additive type. Therefore, a high response to selection should be achievable after several selection cycles. The identification of agronomically superior and anthracnose resistant accessions will be useful in minimising the linkage drag usually breeders come across while transferring disease resistance in already available high yielding but susceptible varieties. This collection represents a valuable genetic patrimony with great potential for the future and as such, germplasm from Himalayan region is an integral part of the secondary diversity of common beans. As a further step, the development of core sets and traits specific sub sets based on this data and subsequent analysis of diversity using more specific molecular markers are recommended to elucidate more information on the overall genetic diversity and particular genes responsible for specific characters of agronomic interest in the present collection.

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