ABSTRACT

Major common bean fungal and viral diseases concurrently occur in farmer's fields leading to complete crop failure when susceptible varieties are grown. Inherent genetic resistance is thus the cheapest source of resistance against diverse pathogenic strains of key diseases affecting common bean as it reduces the need for frequent chemical control application. Deployment of multiple disease resistance bean varieties in farmer's fields would therefore go a long way in reducing yield losses at farm level. Gene pyramiding is one of the breeding methodologies used to develop multiple disease resistant varieties. As a result, pyramided climbing bean populations targeting six major resistance genes (Co-4^2, Co-5 for anthracnose; Phg-2 for angular leaf spot; I and $bc3$ for bean common mosaic necrosis virus and "P.ult" for *Pythium ultimum* root rots) was developed at CIAT, Uganda by hybridizing four parents (G2333 with Co-4^2 and Co-5, Mexico54 with Phg-2, MCM5001 with I and $bc3$ and MLB-4989A with "P.ult") using marker-assisted backcrossing method. The objective of this research was to determine recombination fractions and genetic linkage among key disease resistance genes pyramided, evaluate agronomic qualities of pyramided bean lines and to determine response to selection of key diseases and yield traits of pyramided bean lines based on field evaluations. For linkage analysis, the number of recombinants among linked gene pairs (Co-4^2/Phg-2 and Co-5/"P.ult") was determined by counting Sequence Characterized Amplified Region (SCAR) marker's DNA amplicons on electrophoresis gel pictures of bean lines at both BC_3F_6 and F_2 generations. Recombination fraction r, among gene pairs, the probability of linkage and LOD scores were computed using the statistical relationship of likelihood which assumes a binomial distribution. The agronomic traits of pyramided bean populations were evaluated in four randomized incomplete blocks, with three replicates across two seasons in 2015 at CIAT, Uganda. Thirty seeds were planted per entry in plots of two rows, measuring three meters long, with spacing of 20 cm x 60 cm within and between rows, respectively. Traits evaluated were; days to 50% flowering (DF), number of internodes (Nodeno), number of flower buds per inflorescence (FLB), pods per plant (PDPL), seeds per plant (SPLA), weight of 100 seeds (100SW), growth habit (GH) and flower colour (FLCOL), while seed shape (SS) and primary seed color (PSC) were recorded after harvest. Data was summarized and analyzed for mean comparisons, correlations and trait frequencies recorded. To determine response to selection of common bean genetic pyramids to diseases and yield traits, populations evaluated included pyramided parents, pyramided progenies at BC_3F_9 generation and local checks. The trials were planted in 2016 in Uganda (Kabale and

Kawanda) and northern-Tanzania (Maruku and Kitengule) in randomized incomplete blocks with three replications. Sixty seeds were planted per population per plot in three rows of three meters long, with spacing of 20 cm x 60 cm within and between rows, respectively and climbing beans supported on wooden stakes. Yield traits recorded on all plant stand were; days to 50% flowering, number of pods per plant and weight of 100 seeds. Diseases recorded were angular leaf spot and bean common mosaic virus on leaves and anthracnose on pods using disease rating scale of 1-9. Means of traits were compared using the combined unbalanced ANOVA, while Principal Component Analysis (PCA) was used to account for variations among bean populations. Narrow sense heritability of traits evaluated was computed using parent-offspring regression. Data and appropriate parameters were summarized and incorporated into the breeder's equation ($R = ih^2S$) for computing response to selection. The results were as follows; gene pair $Co-5$/"P.ult" exhibited incomplete linkage (r = 32 cM with a high LOD score of 9.20) in BC_3F_6 population, but relatively stronger linkage in F_2 population (r = 21 cM with a high LOD score of 18.67). However, the linkage among gene pair $Co-4^2$/Phg-2 was very weak in BC_3F_6 population (r = 47 cM with a low LOD score of 0.16) as well as in F_2 population (r = 44 cM with a low LOD score of 0.70). The incomplete and weak linkages found, suggests that each of the four genes above have to be tagged with a specific marker during selection. All agronomic traits evaluated among pyramided bean populations across the two seasons (2015A and 2015B) had significant differences (p<0.05). Gene pyramiding did not affect number of pods per plant, number of seeds per plant and weight of 100 seeds. However, days to 50% flowering, number of internodes and numbers of flower buds formed per inflorescence were significantly different (P<0.05) among pyramids and blank populations (sister lines to pyramids). Transgressive segregation was observed among pyramided progeny lines. Positive and weak correlations (r < 0.5, P<0.01) were observed between number of genes pyramided and agronomic traits evaluated. The mean yield traits of the recurrent parent (G2333) were not yet recovered in segregates by the third backcross, while phenotypic similarity between pyramided lines and recurrent parent was still low for the number of pods per plant (12.5%) and weight of 100 seeds (10.7%). Response to selection was positive for mean days to flowering (five days) and negative for mean number of pods per plant and weight of 100 seeds (-9.5 pods and -1.9 grams respectively). Angular leaf spot and bean common mosaic virus had positive undesirable responses to selection. Pyramided bean lines segregated at BC_3F_9 for diseases and yield traits. The following are conclusions and recommendations; selection with markers tightly linked to disease resistance genes helped to reduce the time for developing bean

pyramided lines. However the dominant SCARs used to develop pyramids and determine genetic linkage among gene pairs, do not differentiate heterozygotes from homozygotes, hence co-dominant markers such as Simple sequence repeats (SSRs) and Single nucleotide polymorphisms (SNPs) are recommended for future gene pyramiding in beans. Agronomic traits of progenies of pyramided bean lines were still segregating at the BC_3F_6 generation and could be advanced further and fixed in late generations such as BC_3F_{10}. Likely yield penalties associated with gene pyramiding reported in literature should be tested using fixed bean populations. The superior lines identified during the study, such as G63, G65 and G66 need further testing using multi-location trials and farmer's fields to assess their adaptability, so as to release them as commercial varieties.