# GENETIC DIVERSITY AND POPULATION STRUCTURE OF COMMON BEAN GENOTYPES USING MORPHOLOGICAL TRAITS AND SSR

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## ABSTRACT

**The objectives of this study were to estimate the performance of the common bean (*Phaseolus vulgaris* L.) genotypes under water-stress conditions, and their genetic diversity. White bean surpassed the others for relative water content, root/shoot ratio and leaf area under water-stress condition. Scatter plot indicates a strong association of yield with pod numbers plant$^{-1}$, branch number and harvest index. A total of 69 polymorphic were obtained, applying 26 SSR primers on 14 genotypes. Major allele frequency was 0.601, and the average value of PIC was 0.407. The highest value of gene diversity (0.745) and PIC (0.704) were recorded for BMd-23 marker. Molecular variance among population indicated 25%, while 47% was realized within populations. Structure analysis divided the common bean genotypes into three groups (DeltaK value =3). Chity and Boschbohnen were identified to have a mixed ancestor while all the others were pure at their populations. A dendrogram and PCoA analyses are accordingly indicated three groups of the genotypes based on SSR marker data. STRUCTURE, UPGMA and PCoA analysis revealed the presence of two separated gene pools of Andean and Mesoamerican common beans, with a high level of genetic differentiation ($F_{ST}$ value=0.250). Both phenotypic and molecular genetic outcomes here would accelerate future improvement programs.**

**Keywords: water stress, polymorphic information contents (PIC), principal component analysis (PCA), allele frequency**



# التنوع الجيني والتركيب المجتمعي لانواع من الفاصوليا العادية (*Phaseolus vulgaris* L.) باستعمال الصفات المورفولوجية و SSR

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## المستخلص

فاصوليا العادية هي ضمن محصول بقوليات البذرية ذاتي التلقيح (2n=2x=22)، تستخدم كمصدر غذائي جيد للانسان. أهداف الدراسة كانت تقدير أداء الطروز الوراثية للفاصوليا تحت ظروف الجهد المائي، وكذلك تقدير تنوع الوراثي. تفوق White bean على الأنواع الأخرى من حيث المحتوى المائي النسبي ونسبة الجذر/ساق ومساحة الأوراق تحت ظروف الجهد المائي. تم الحصول على مجموعة 69 أليلاً متعدداً، باستخدام 26 بادئة SSR على 14 تركيبا وراثيًا. كان تردد الأليل الرئيسي 0.601 ، ومعدل PIC كانت 0.407. تم تسجيل أعلى قيمة للتنوع الجيني (0.745) و PIC (0.704) لبادئة BMd-23. أشار التباين الوراثي بين االمجتمعات إلى 25٪ ، بينما تحقق 47٪ بين الافراد الجتمع الواحد. قسم تحليل بنية الأنماط الجينية التراكيب الوراثية إلى ثلاث مجموعات (قيمة DeltaK = 3). تم تحديد التركيبين الوراثيين Chity و Boschbohnen على أنهما يتميزان بسلف مختلط بينما كان الآخرون نقيين في مجتمعاتهم ، وبناءً على ذلك ، تمت الإشارة إلى ثلاث مجموعات من الأنماط الجينية من خلال تحليلات مخطط الشجرة و PCoA بناءً على مصفوفة الاختلاف لـ SSR. كشف تحليل STRUCTURE و UPGMA و PCoA عن وجود مجموعتين من الجينات المنفصلة من فاصوليا الأنديز وأمريكا الوسطى، مع مستوى عالي من التمايز الجيني (قيمة F=0.250). كل من النتائج المظهرية الوراثة الجزيئية هنا من شأنها تسريع برنامج التحسين المستقبلي للفاصوليا العادية.

**الكلمات المفتاحية: الإجهاد المائي، محتوى المعلومات متعدد الأشكال (PIC)، تحليل المكون الرئيسي (PCA)، تردد أليل**

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Common bean (*Phaseolus vulgaris* L.) belongs to Leguminosae family, with a small genome size of 580 Mbp (44). It is considered among the most important crops in the world, plays an imperative role in sustainable agriculture (5, 6, 23). The plant is a predominantly self-pollinated crop plant (39), grown and consumed in various Asian countries (27). In Iraq it is cultivated for both green pod and dry seed (20). Common bean performs the best in moderate growing temperatures (>10 °C and <30 °C) with about 400 mm of annual precipitation. Various biotic and abiotic factors limit the productivity of the common bean, as a reduction of 88% in common bean yield has been observed due to severity of drought (24). Morphological characterization was the marker systems used in genetic diversity (52). Low level of polymorphism and heritability, late expression of morphological markers, and their influences by the environment are from their limitations for the accurate estimation of genetic relationship between the studied genotypes (42). New technologies such as DNA markers, parallel to morphological markers, enhancing the efficiency of selection in the breeding program (26, 19). Genetic diversity in common bean has been studied using different molecular markers such as RFLP, RAPD and AFLP (53). While, SSR marker has several advantages over most of others for genetic characterization, being highly polymorphic and reproducible, enormous extent of allelic diversity, co-dominant and highly reproducible, distributed across the genome (22). SSR markers were exploited successfully to reveal genetic variation among common bean genotypes and identify their relatedness (36). The objectives of this study were to investigate common bean genotypes for morphological traits under normal and water stress conditions and to determine the genetic diversity and structure analysis of the genotypes based on SSR markers.

## MATERIALS AND METHODS

**Plant materials:** Fourteen common bean genotypes, were used which obtained from agricultural research centers in Kurdistan, Iraq. They were planted on 11th March 2021 in Chwarqurna field, south-west of Ranya province with the latitude 36° 6' 13" N and the longitude 44° 49' 22" for elevation of 545m. Seeds of 14 common bean genotypes were sown within rows of three meters length and 50 cm between the rows apart ($1.5m^2$), distance between the plants within row was 30 cm. A randomized complete block design was used with three replicates. Irrigation was conducted twice a week and all other necessary managements were applied equally on all the plots. Data was recorded for the growth characteristics, yield and its components, and statistically analyzed by using statistical program package "XLSTAT 2016.4.01.20780". One-way analysis of variance (ANOVA) was followed by Duncan multiple range tests to compare the means of the traits at the probability level of 5%. A distance biplot analysis derived from principal component analysis (PCA) was conducted for Pearson (n) type (55), to discriminate between different genotypes on the basis of studied characteristics.

**Drought stress experiment**

An experiment was conducted under greenhouse conditions at plant nursery belongs to Ranya Municipality in Oct. 2021, to assess the effect of water stresses on the performance of the common bean genotypes, using CRD experiment with three replicates. The seeds were planted in plastic pots. Three irrigation regimes with control (Normal watering), 1st level of drought (watering every five days until maturation) and 2nd level of drought (watering every two weeks until maturation) were followed. After 35 days data was recorded for plant height, number of tillers/plant, relative water content (33), root/shoot ratio, and leaf area (14). Collected data was subjected to analysis of variance (ANOVA), mean values among water stress treatments were determined and the means compared according to LSD test at 5% significant level.

**DNA marker assay**

DNA was extracted from three weeks leaves, using *Quick*-DNA Plant/Seed Miniprep Kit. Forty-five SSR primers of common bean were used in this study (Table 1).

**PCR reaction and gel electrophoresis** Molecular works were carried out at Molecular Laboratory, Department of Medical Science, College of Science, University of Raparin**.** All

PCR amplifications were carried out in a standard PCR machine (techne prime thermal cycler, UK). The amplification program was set up with the following reaction: run with a hot start of 92 $^{o}$C for 5 min; then 40 cycles of 92 $^{o}$C denaturing for 1 min; 47-57 $^{o}$C annealing for 1 min and 72 $^{o}$C extension for 2 min; followed by a 5-min final extension at 72 $^{o}$C. Size of PCR reaction mix was 20µl, contained 10µl of amaR 2X PCR mix, 3µl of DNA, 2µl forward, 2µl reverse and 3µl double distilled water. An amount of 5µl aliquot of the amplified PCR product from each genotype was mixed with 0.5µl of electrophoresis 6x loading dye, then analyzed by gel electrophoresis at 2% agarose in 1.0 × TBE buffer (Sambio). Agarose gels were stained with safe stain (cleaver scientific, UK) with the rate of 4µl for 250 agarose gel, before pouring into the electrophoresis tray. The gels were run on 90V for 120 minutes, and separated fragments were visualized under UV translaminator using electrophoresis Gel Doc (MultiDoc-It Imaging System by UVP).

**Table 1. List of SSR markers, sequences, and annealing temperature used to screen 14 common bean genotypes**

| Marker | Motif repeat | Forward primer/Reverse Primer | Annealing temp. | Source |
|---|---|---|---|---|
| BMd-20a | (TA)5 | GTTGCCACCGGTGATAATCT/GTGAGGCAAGAAGCCTTCAA | 47 | |
| BMd-20b | (AT)5 | CCGTTGCCTGTATTTCCCCAT/CGTGTGAAGTCATCATCTGG AGTGGTC | 47 | |
| BMd-23 | (GA)5 | GGCTTGGTCCTCTCATTGAA/ TGGAAATTACCACCATGCAA | 47 | (9) |
| BMd-30 | (TTAA)3 | CAGCAAATGCAACGCTAAGA/GGTTGAATTTTGAAACCCTGA | 47 | |
| BMd-50 | (AAC) | TGGTGAGAGAAGGACAATAGCA/GCCGCTTGTGACGTTTATT T | 47 | |
| M18094 | (CCA)5 | TAATTTCTCTCTTCCCATCCCAAAC/ GTAGTAATAAGGAGGAGGCGGTGAG | 47 | |
| M18093 | (CCA)6 | CCAGCTACCATCTCCTCCATCGL/ TAGTGGTGGAGGTGGAGATTT | 47 | (58) |
| U18791 | (TA)22 | GGGAGGGTAGGGAAGCAGTA/ GCGAACCACGTTCATGAATGA | 47 | |
| (CV53739) | - | TGGCCGTACAACTGGTATTG/ GCTCTGCAGATGTGGTGAAA | 47 | (28) |
| PCAMTA 1 | - | TGGTTGATGGTGTGGAGGAT/ GTGTCTGGTCGAAAT CCACG | 57 | (45) |
| P23 | (AC)7 | AGGAGCTGGAGCTGTAAGCA/ GCCGTGCTAGTGAAACGAAT | 47 | |
| Ph2 | (TC)16 | CCCTTTGCTCCTTCTGTCCT / GATTGGAGAGCGGATTGGTA | 47 | |
| Ph3 | (TA)6 | CTAAGATCCCCAAGGCAACA/ TACAGGCACACGATGGAAAA | 47 | Newly developed |
| Ph4 | (AC)7 | GCCCTTAAAGATGTGGTTCC/ GTGAAGAGGGGTTGCACAGT | 47 | |
| Ph5 | (TG)5 | GGTTTCAGTTGGCGGATTTA/ ACCCAATCCACACGGTACAT | 47 | |
| Ph6 | (TTTA)4 | CCAAACCCAATAATACTAGAGGTGA/ GCGTTACCAGACCAGATGCT | 47 | |
| Bmd17 | (CGCCAC)6 | GTTAGATCCCGCCCAATAGTC/CAACAAACGGAAGGGCGTG GTTT | 47 | (18) |
| Vm71 | (AG)12(AAAG)3 | TCGTGGCAGAGAATCAAAGACAC /TGGGTGGAGAAAACAAACC | 47 | |
| BMc121 | (GA)19 | TGCATTCACCGCTATTACGA /CACTGTAGCCACCATGAGCA | 47 | |
| BMc124 | (GA)12 | TGTCGGTTGTGAGACAGGAG /TTGGAGCTGCTACTCCCACT | 47 | |
| BMc125 | (CT)5 | GTTGCAATTCATCACCATGG /GCAGTGGGAGGGTATTTTG | 47 | |
| BMc128 | (TAA)5 | AATAACTGAGCAATAGAATGCCTAA /AAAGGGTCGATTGTGACTGTG | 47 | |
| BMc132 | (GT)7 | GCTTACAACTTTACACACTCCTATG /GAAGCTGGTGGTGTTTTAATGG | 47 | (10) |
| BMc171 | (CT)11 | CCTTTCACTTCACTTGTGGTTC /GCCATGGCTGATTCAGTAGC | 47 | |
| BMc184 | (ATC)7 | GCAGTTCGATTAACGGAGAG /GCCCATATGTGTGGAGTTGA | 47 | |
| BMc187 | (GAG)8 | GAGCAAGAGTCCTCATCACG /GTGGGCTCGTTCTCGTTG | 47 | |

**Molecular data scoring and analyses**

The amplified fragments of the primers' alleles were scored as "1" and "0" for the presence and absence of alleles, respectively. Polymorphism percentage, gene diversity and polymorphic information content were estimated on the basis of frequencies of identified alleles. POPGENE v1.32 software was used to determine the allele frequency, Na, Ne and gene diversity per locus, for each primer. STRUCTURE 2.3.4 software was used to determine structure analysis. The run parameters set up as 100,000 burn-in periods and 100,000 Markov Chain Monte Carlo

(MCMC) replication. The K value set up from 2 to 10 and 10 replicate runs were performed for each value of K. To select the best number of K (subpopulation), Structure Harvester was used. A dendrogram of common bean genotypes was generated based on UPGMA method via Power Marker v3.25 and then visualized using MEGA X (31). GenAlEx V6.5 was implemented to calculate principal coordinates analysis (PCoA) and Analysis of Molecular Variance (AMOVA).

## RESULTS AND DISCUSSION

### Agro-morphological characteristics

Significant differences were found among 13 common bean genotypes (except Brazilian flat bean) for all the growth characteristics, yield and its components at 1% probability level. The presence of high genetic distance between the genotypes was indicated. Red bean genotype shows the highest plant height (53cm) when compared to the others (Table 2). In terms of branch number, the maximum value (11.67/plant) was recorded for Straik genotypes, having low plant height. Chity genotype had the maximum value of first pod height (25cm), followed by Gold life. This trait could be considered in a plant breeding programs, to facilitate mechanical harvesting of crop after maturation (60). Optimal timing of transition from vegetative to reproductive stage considered a key adaptive characteristic in common bean, making flowering initiation study to be highly important (7). Appearing of first flower in a very short period (34 days) refers to Dwarf French bean genotype and was matured (DTM) in 84 days after sowing. This genotype seems to have the longest flowering and fruit setting period; however, it did not very desired for seed yield. However, some of the genotypes bloom slowly but mature fast as compared to other genotypes, such as: Duru (DTF: 50; DTM: 84) and Gold life (DTF: 53; DTM: 84). Some of the genotypes bloomed in a much shorter period, while days to maturation were quite long, such as Dwarf bean sunray, Red bean, and Euro. Studying these characteristics could be important for legume crops, especially in the growing season arid and semi-arid climates (2). The highest seed yield (SY) was obtained by Dwarf bean sunray (14857.5 kg/ha; 111g/plant) with 4.3 number of seeds per pod (NOS/pod) and 9 pods/peduncle, followed by Straik (11360.4 kg/ha; 85.4 g/plant) with 5.3 NOS/pod and the highest number of pod/plant (74.7) and harvest index (81.28). Straik genotypes was highly branched (11.3 BN/plant), followed by Black horse. These results can be further clarified in Tables 3. High variations in growth, seed yield and its components of common bean genotypes are in accordance with the results of other researchers (35).

**Table 2. Mean comparison of some growth characteristics among 13 common bean genotypes, grown in Chwarqurna, Sulaimani (2020-2021). The comparisons were made using Duncan's multiple range test at 5 % level of probability**

| Genotypes | Plant height (cm) | Branch number/ plant | First pod height (cm) | Days to first flowering | Flowering period (d) | Days to maturity |
|---|---|---|---|---|---|---|
| Black horse | 40.000 d | 10.33 ab | 11.67 e | 48.0 d | 59.0 h | 100.0 c |
| CHAMGOTA | 41.000 d | 5.67 e | 7.67 f | 49.0 d | 71.0 b | 102.0 b |
| Duru | 35.000 e | 5.33 ef | 11.00 e | 50.0 c | 58.3 h | 84.0 e |
| Euro | 40.667 d | 8.67 bcd | 8.67 f | 40.0 g | 75.7 a | 102.0 b |
| Dwarf bean sunray | 35.000 e | 7.67 d | 4.67 g | 53.7 b | 70.3 b | 109.0 a |
| Boschbohnen | 20.667 g | 9.67 bc | 8.00 f | 43.0 f | 72.3 b | 95.0 d |
| Red bean | 53.667 a | 4.00 ef | 18.00 c | 44.0 e | 66.7 cd | 102.0 b |
| Chity | 44.667 c | 5.00 ef | 25.00 a | 39.0 g | 63.0 fg | 84.7 e |
| White bean | 50.000 b | 8.00 cd | 17.00 c | 48.7 d | 64.0 efg | 110.0 a |
| Straik | 34.000 e | 11.67 a | 5.00 g | 39.0 g | 67.7 c | 85.7 e |
| Gold life | 52.000 a | 3.67 f | 20.00 b | 53.0 b | 65.7 cde | 84.0 e |
| Dwarf French bean | 30.000 f | 5.00 ef | 5.33 g | 34.3 h | 65.0 def | 84.0 e |
| Shaker | 35.000 e | 5.67 e | 14.33 d | 55.0 a | 62.0 g | 95.7 d |

**Values within each column that do not share a common letter are significantly different by Duncan's test at P ≤ 0.05**

Gold life genotype had the highest value of NOS (5.3 pods/plant) and root weight (41.67 g/plant) with all setting flowers. However, the genotype had recorded very low seed yield, the

other desired characteristics could be used in an improvement breeding program of common bean. Euro genotype had recorded the highest weight of plant biomass; while it behaves low value for almost other characteristics. Genetic architecture of these genotypes for source-sink dynamic in exhibiting low remobilization of assimilates toward the seed yield could be a reason of other traits' performance (50). According to the current results, there are significant differences between genotypes in number of seeds per plant, plant height, first pod height, non-podded flower, 100-seed weight, root weight, plant biomass, and seed yield. Due to the increased demand for common bean plants in traditional farming, there is a high degree of diversity among common bean plants, which is characterized by low selective pressure. Other researchers (40) who work with common bean plants have realized genotype-specific variation among the plants. Differences in grain yield between small-seeded and large-seeded common beans are due to genetic adaptation of the distinct gene pool to the region of domestication rather than to environmental factors (41). Large seed size indicates a high nutrition quality of the seeds and their germination, which in turn designates high genetic potential (37).

**Table 3. Mean comparison for yield and its components among 13 common bean genotypes, grown in Chwarqurna, Sulaimani (2020-2021). Means were compared using Duncan's multiple range test at 5 % level of probability**

| Genotypes | Pod/ number plant | Pod number/ peduncle | Number of seed/pod | Number of empty pods | Seed yield (g/plant) | 100. seed weight | Root weight | Plant biomass | Harvest index | Seed yield (kg/ha) | Non-podded flower |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Black horse (G1) | 53.7 c | 5.0 cde | 4.3 abcd | 4.7 bc | 54.80 e | 24.70 fg | 14.33 c | 140.67 gh | 38.80 e | 7306.2 e | 0.0 c |
| CHAMGOTA (G2) | 26.3 g | 4.3 e | 3.7 cdef | 1.7 f | 22.31 g | 23.52 g | 6.00 e | 353.00 b | 6.32 k | 2975.1 g | 41.0 a |
| Duru (G3) | 42.3 e | 7.7 b | 3.0 efg | 5.7 ab | 41.64 f | 34.48 c | 11.00 d | 235.67 de | 17.70 h | 5552.2 f | 0.0 c |
| Euro (G4) | 45.7 d | 4.7 de | 3.7 cdef | 1.7 f | 52.40 e | 27.66 e | 42.33 a | 436.67 a | 12.00 i | 6985.7 e | 6.0 b |
| Dwarf bean sunray (G5) | 72.3 b | 9.0 a | 4.7 abc | 6.3 a | 111.48 a | 30.71 d | 11.00 d | 255.33 d | 43.66 d | 14857.6 a | 4.7 b |
| Boschbohnen (G6) | 41.0 e | 4.0 e | 4.0 bcde | 3.7 cd | 58.87 d | 37.40 b | 14.67 c | 37.67 j | 56.45 c | 7844.9 d | 3.7 bc |
| Red bean (G7) | 25.3 gh | 6.0 cd | 2.7 fg | 3.3 de | 14.10 i | 31.27 d | 11.00 d | 158.67 fg | 8.88 j | 1875.6 i | 0.0 c |
| Chity (G8) | 37.0 f | 6.0 cd | 4.7 abc | 2.0 f | 67.49 c | 37.53 b | 10.33 d | 104.00 hi | 65.74 b | 8999.1 c | 3.3 bc |
| White bean (G9) | 38.3 f | 5.0 cde | 2.3 g | 1.0 f | 42.73 f | 54.29 a | 14.67 c | 189.67 f | 22.49 f | 5696.9 f | 0.0 c |
| Straik (G10) | 74.7 a | 6.3 c | 5.3 a | 2.3 ef | 85.20 b | 23.05 g | 10.00 d | 104.67 hi | 81.28 a | 11360.4 b | 2.3 bc |
| Gold life (G11) | 14.3 j | 4.7 de | 5.3 a | 5.0 ab | 20.89 gh | 25.60 f | 41.67 a | 310.00 c | 6.73 k | 2784.9 gh | 0.0 c |
| Dwarf French bean (G12) | 21.7 i | 4.3 e | 3.3 defg | 1.7 f | 19.02 h | 31.12 d | 5.33 e | 92.67 i | 20.53 g | 2536.4 h | 0.0 c |
| Shaker (G13) | 24.0 h | 5.0 cde | 5.0 ab | 5.0 ab | 23.56 g | 19.18 h | 18.33 b | 200.00 ef | 11.78 i | 3140.9 g | 3.7 bc |

**Values within each column that do not share a common letter are significantly different by Duncan's test at P ≤ 0.05.**

## Water stress experiment

Plant physiology and morphology are altered by water stress, which varies depending on the degree and duration of exposure (21). Drought stress is more severe than other abiotic stresses that negatively impacts yield components of plant species in arid and semi-arid regions (3, 30). Analysis of variance indicating high significant differences among the genotypes under drought stress for plant height, relative water contents and leaf area, indicating wide variation among the common bean genotypes (Table 4). The effect of drought stress condition on the performance of 13 common bean genotypes, indicates wide distance in the performance of these genotypes based on the characteristics recorded. The highest plant height under water stress conditions refers to Red bean (G7). This genotype had also recorded the highest leaf area of 60.67 $cm^2$. Relative water content of the plants varies greatly among the genotypes. Chity genotype is indicated to have the highest mean value of water content (77.67%), followed by genotype Euro (76%). Red bean genotype had the lowest water content record. White bean had the highest desire values for all the traits studied here except plant height. Relative water

content (RWC) of leaves is a useful measure of the water status of plants, and it is significantly altered by drought. This trait is highly recommended to evaluate the ability of genotypes in retaining water under water deficit conditions (32). In terms of the physiological consequences of a cellular water shortage, RWC is probably the most relevant indicator of plant water status. Water stress inhibits leaf production and promotes senescence and abscission in plant (43), resulting in a reduction in the overall amount of leaf area produced by plant. Base on the above facts, White bean genotype seems to have the best performance under the water stress condition of semi-arid area in Kurdistan. The finding is also consistent with those of Thinley and Dorji (51) on cowpea, who found that a drop in LA was detected in cowpea during water stress, trying to reduce the rate of transpiration and surface area exposition to radiation caused by a water deficit. This trait could be further investigated in the future studies to emphasize the better rooting system of common bean under water stress conditions. Larger root size resulting in their higher assimilation of nutrients (48). Water stress has been linked to a decrease in leaf production as well as an increase in leaf senescence and abscission (56), which may serve as a drought-avoidance mechanism. The genotypes in general had better perform when interacted with the normal watering, while genotype White bean interacted with the second level of stress had better performance in terms of root/shoot ratio.

**Table 4. Effect of the genotypes and different water stress conditions on some early growth characteristics studied on 13 common bean genotypes**

| Experimental factor | | Plant height | Relative water contents | Root/shoot | leaf area |
|---|---|---|---|---|---|
| genotypes | Black horse (G1) | 23.33 e | 71.667 cde | 0.102 bc | 36 ef |
| | CHAMGOTA (G2) | 21 f | 73.778 abcde | 0.078 fg | 34 f |
| | Duru (G3) | 29.67 c | 75 abcd | 0.09 | 42.667 d |
| | Euro (G4) | 22.67 ef | 76 ab | 0.093 cde | 46.556 c |
| | Dwarf bean sunray (G5) | 21.67 ef | 74.333 abcde | 0.076 g | 42.333 d |
| | Boschbohnen (G6) | 16 h | 74 abcde | 0.096 bcde | 48.333 c |
| | Red bean (G7) | 40.33 a | 70.667 e | 0.089 def | 60.667 a |
| | Chity (G8) | 36.33 b | 77.667 a | 0.108 b | 58 ab |
| | White bean (G9) | 36.33 b | 76.556 ab | 0.145 a | 60.667 a |
| | Straik (G10) | 26.67 d | 73.333 bcde | 0.091 | 40.667 d |
| | Gold life (G11) | 37.11 b | 73.333 abcde | 0.084 efg | 40.667 d |
| | Dwarf French bean (G12) | 17 gh | 71 de | 0.075 g | 55.333 b |
| | Shaker (G13) | 18.11 g | 75.333 abc | 0.099 bcd | 39.333 de |
| LSD value 0.05 | | 2.046 | 2.433 | 0.007 | 2.245 |
| water stress | Control | 30.82 a | 81.84 a | 0.11 a | 58.07 a |
| | Stress level 1 | 26.74 b | 74.82 b | 0.086 b | 43.84 b |
| | Stress level 2 | 22.33 c | 65.56 c | 0.085 c | 37.74 c |
| LSD value 0.05 | | 0.983 | 1.169 | 0.004 | 1.078 |

**Association among the common bean genotypes**

PCA analysis was performed for the 13 common bean genotypes based on the agro-morphological characteristics under normal environmental condition (Figure 1). A total of 16 phenotypic traits were used to construct a two-dimensional scatter plot. Relationships among different parameters were displayed in the graph based on PCA1 and PCA2, for the rank correlation matrix. The first two PCs revealed nearly half of the total morphological variation (46.53%) among the evaluated genotypes. The result still suggests that there is a need for a higher number of components to explain the variation among the genotypes more effectiveness (38). The cosine of the angle between the vectors of different characteristics approximates their associations. Seed yield was in strong and positive association with pods number per plant and peduncle, branches number and harvest index, while number of seeds pod$^{-1}$ is in a strong negative association with 100-seed weight. Theses relationship could be realized clearly on the biplot diagram. Indeed, the performance of the genotypes for different traits is indicated also here, Dwarf bean sunray performs better than other genotypes in terms of the yield and some its components under field condition. Dwarf bean sunray and Straik genotypes were separately concentrated on the left side of the

plot, having a clear boundary with the other groups. A group of six genotype (Balck horse, Duru, Euro, Boschbohnen, Chity and White bean) concentrated around the center of the plot, being away from the genotypes on the right side with a few outlined clustering.

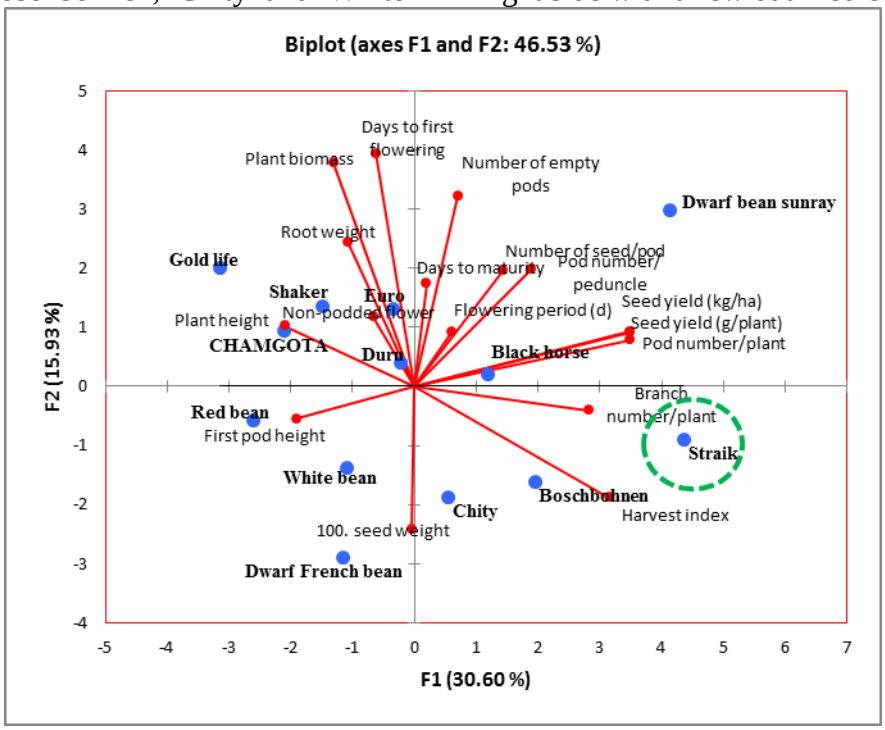


**Figure 1. Two-dimensional PCA scatter plot of the 13 common bean genotypes baes on the agro-morphological traits**

**Molecular data analysis**

Parameters of genetic diversity of 14 common bean genotypes were examined using 26 SSRs out of the total 45 DNA markers. They were effectively used here, and able to distinguish between the genotypes. Other researchers have also investigated that SSR loci provide excellent distinguishing sites across closely related species (36). SSR markers are less affecting by the environment is among the advantages of widespread applying for molecular breeding of different species (8). They become the method of choice in genetic diversity research of common bean (17). A total of 69 alleles from 26 polymorphic primers were obtained when applied on 14 common beans (Table 5). Allele numbers ranged from 2 alleles/ primer to 6 alleles (BMd-23). While effective number of alleles (ne) ranged from 1.415 (BMc125) to 3.920 alleles (BMd-23) with an average of 2.082 alleles. The major allele frequency ranged from 0.357, for BMd-23, to the highest value of 0.821 for BMc125 and BMc171 with the mean value of 0.601. The maximum value of gene diversity was showed by BMd-23 (0.745) followed by BMc121 (0.648) and CV53739 (0.648). The polymorphic information content (PIC) value ranged from 0.250 (BMc125) to 0.704 (BMd-23), with an average of 0.407. The mean value of alleles per locus (2.65/primer) is reasonable, however higher alleles per locus were investigated by other researchers (46, 54). In contrast, the effective number of alleles in this investigation was 2.08 alleles per locus were notably higher when compared to 1.89 alleles per locus by Gioia, et al. (23). Indeed, the highest major allele frequency (0.821) was obtained from SSR markers BMc125, while the minimum value of 0.357 was obtained from SSR marker BMd-23. Gene diversity and PIC were expressed reversely to the allele frequency. Here, MBc-23 marker is more specified with common alleles rather than rare alleles in the population of common bean, indicating no satisfaction in the allelic saturation for the current population in terms of the current marker (1). PIC valued a range from 0.250 to 0.704 for the markers BMc125 and BMd-23, respectively. Different pattern of PIC values has been reported previously on common (13, 24). In general, the applied SSR markers in this study were informative in distinguishing the tested genotypes based on PIC value (4). The markers identified with high gene diversity

and PIC could be useful for the conservation of poorly characterized common bean and determining the extent of the gene pool for this crop.

**Table 5. Allele frequency parameters generated by 27 SSR markers on 14 common bean genotypes**

| Marker | Na* | Ne* | Major allele frequency | Gene diversity | PIC |
|---|---|---|---|---|---|
| BMd-20a | 2 | 1.774 | 0.679 | 0.436 | 0.341 |
| BMd-20b | 2 | 1.849 | 0.643 | 0.459 | 0.354 |
| BMd-23 | 6 | 3.920 | 0.357 | 0.745 | 0.704 |
| BMd-30 | 2 | 1.960 | 0.571 | 0.490 | 0.370 |
| BMd-50 | 2 | 1.690 | 0.714 | 0.408 | 0.325 |
| M18094 | 2 | 1.800 | 0.667 | 0.444 | 0.346 |
| M18093 | 2 | 1.742 | 0.692 | 0.426 | 0.335 |
| U18791 | 3 | 1.894 | 0.679 | 0.472 | 0.409 |
| CV53739 | 4 | 2.840 | 0.423 | 0.648 | 0.578 |
| P CAMTA1 | 2 | 1.980 | 0.550 | 0.495 | 0.372 |
| P23 | 2 | 1.988 | 0.538 | 0.497 | 0.374 |
| Ph2 | 3 | 2.579 | 0.429 | 0.612 | 0.530 |
| Ph3 | 3 | 2.010 | 0.607 | 0.503 | 0.407 |
| Ph4 | 2 | 1.849 | 0.643 | 0.459 | 0.354 |
| Ph5 | 2 | 1.960 | 0.571 | 0.490 | 0.370 |
| Ph6 | 2 | 1.849 | 0.643 | 0.459 | 0.354 |
| Bmd17 | 2 | 1.774 | 0.679 | 0.436 | 0.341 |
| Vm71 | 3 | 2.667 | 0.500 | 0.625 | 0.555 |
| BMc121 | 4 | 2.841 | 0.464 | 0.648 | 0.582 |
| BMc124 | 3 | 1.931 | 0.643 | 0.482 | 0.395 |
| BMc125 | 2 | 1.415 | 0.821 | 0.293 | 0.250 |
| BMc128 | 2 | 1.960 | 0.571 | 0.490 | 0.370 |
| BMc132 | 2 | 1.690 | 0.714 | 0.408 | 0.325 |
| BMc171 | 3 | 1.436 | 0.821 | 0.304 | 0.274 |
| BMc184 | 4 | 2.579 | 0.500 | 0.612 | 0.541 |
| BMc187 | 3 | 2.142 | 0.500 | 0.533 | 0.424 |
| Mean | 2.654 | 2.082 | 0.601 | 0.495 | 0.407 |
| St. Dev | 0.9774 | 0.537 | | 0.537 | |

*** na = Observed number of alleles, ne = Effective number of alleles, PIC=Polymorphic information content**

## Structure analysis

Structure Harvester showed that the maximum DeltaK value was K=3 (3 subpopulation), dividing the 14 common bean genotypes into three populations [Population1 (red), Population2 (green) and Population3 (blue)]. The results were selected from STRUCTURE (Figure 2). In population1 five genotypes [Red bean (G7), Dwarf French bean (G13), Shakar (G14), Straik (G10) and Chity (G8)], five from population2 [Euro (G4), Duru (G3), Chamgota-Kidney bean (G2), Dwarf bean sunray (G5) and Black horse (G1)] and in population3 four genotypes [Gold life (G11), White bean (G9), Brazilian flat bean (12) and Boschbohnen (G6)] were structured. The genotypes within populations were classified as pure for those that inferred ancestry based on probability score more than 0.80 and admixture with less than 0.80. All genotypes in the populations were identified as pure, except Chity (G8) that presented as admixture in population1 and Boschbohnen (G6) was identified as admixture in population3. These two genotypes could have a mixed ancestry as clear from their different color shared by parents from other gene pools.

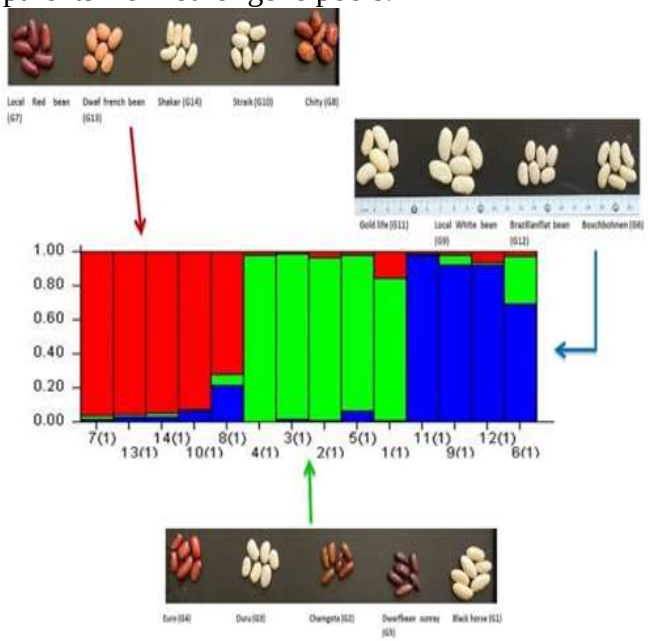


**Figure 2. Population structure of 14 common bean genotypes based on 26 SSR markers. Genotype membership to the three clusters**

The results obtained are in accordance with the finding of Scarano, et al. (47), who reported three sub-populations of 25 common bean populations, using 10 SSR markers. Also, Zargar, et al. (59) classified 51 common bean genotypes into three groups using 23 SSR markers. In addition, Carović-Stanko, et al. (12) revealed three clusters of Croatian common bean landraces using 26 SSR markers. But others researchers (25, 46, 54) revealed a clear structure analysis of studied common bean into two groups, corresponding the main gene pools of Mesoamerican and Andean.

## Genetic diversity based on the SSR data

PowerMarker (34) was used to determine the dissimilarity matrix for 14 verities of common bean and UPGMA dendrogram was visualized

using MEGA X (31). The dendrogram (Figure 3), based on the dissimilarity matrix values, shows a clear picture of the relatedness among studied genotypes.

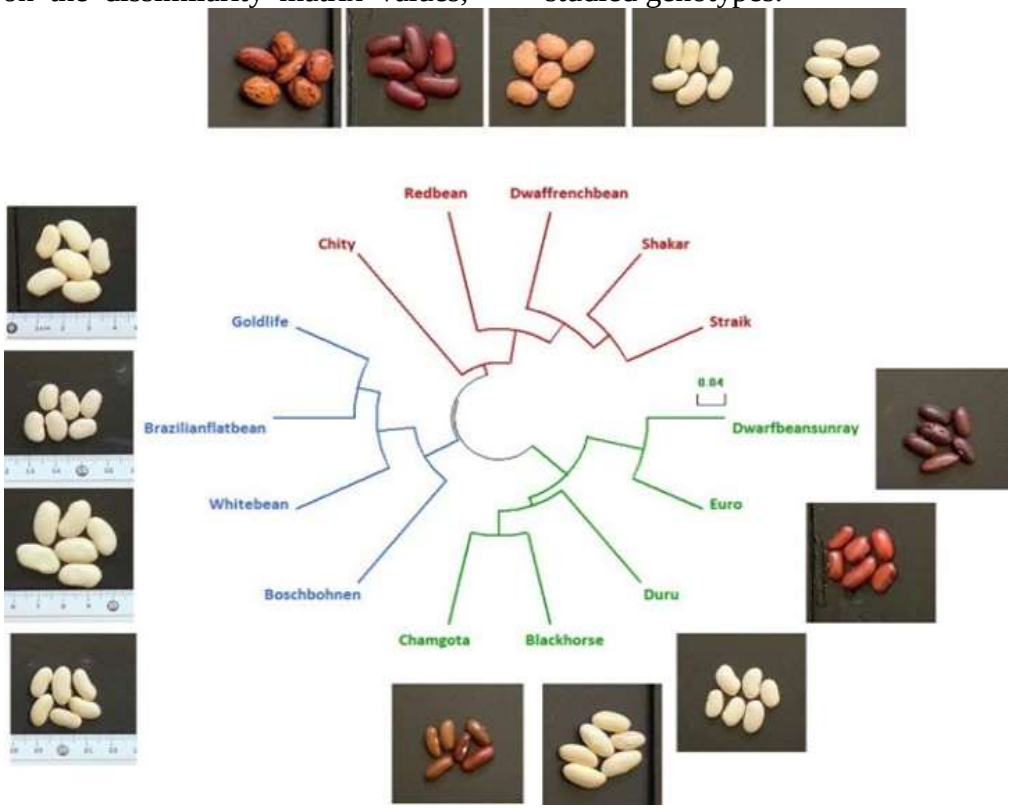


**Figure 3. Dendrogram showing the genetic relatidness among 14 common bean genotypes based on SSR data**

Three clusters; in red, green and blue color were observed. The red cluster1 consisted of five genotypes; Red bean (G7), Dwarf French bean (G13), Shakar (G14), Straik (G10) and Chity (G8). While, Euro (G4), Duru (G3), Chamgota-Kidney bean (G2), Dwarf bean sunray (G5) and Black horse (G1) were in green cluster. Finally, Gold life (G11), White bean (G9), Brazilian flat bean (12) and Boschbohnen (G6) were grouped together in the blue cluster. The dendrogram revealed a close relationship between the red and blue clusters. The UPGMA analysis shows same sub-grouping in each cluster and they also found it to be in accordance with structure analysis. It has been reported that the common bean has two major gene pools, Mesoamerican and Andean (15, 38). Same pattern could be realized in the current study, representing Andean gene pool, contains white, yellow and red kidney of medium and large seeds, and Mesoamerican gene pool of the green cluster, that includes black and small seeds bean. Both gene pools differentiate agronomic traits such as seed size, weight and color. Also, the dissimilarity matrix values ranged from 0.221 (between Euro and Dwarf bean sunray) to 0.703 (between Gold life and Read Bean). In this study, the Andean genotypes (red and blue) represent 64.29% of the total genotypes evaluated and Mesoamerican (green) represents 35.71%. The principal co-ordinates analysis (PCoA) of 14 common bean genotypes using 26 SSR markers revealed similar results as observed by UPGMA based clustering. The PCoA revealed the diverse distribution of the genotypes on the coordinates (1 vs. 2) plot (Figure 4). The Cluster C1 was located in quadrant 1 except the 'Black' genotypes. Cluster C2 distribute in quadrant 2 and 3. While cluster C3 were in quadrant 3 and 4. As well as the subpopulations C2 and C3 were closely related than C1, which agrees with the phylogenetic tree output. The principal co-ordinates analysis accounted for 55.90% of the total variation on the three first principal coordinates. The genotypes distributions were 22.46%, 20.23% and 13.21 for the first, second and third principal coordinate, respectively. The PCoA analysis confirmed the results of STRUCTURE and UPGMA analysis. Both gene pools were almost completely separated by the center of the vertical axis of PCoA. In

addition, both admixture genotypes Chity (G8) in population1 and Boschbohnen (G6) in population3 were between Andean and Mesoamerican gene pools. It has been reported that the accuracy of STRUCTURE, UPGMA and PCoA analysis are comparable and informative for investigating the genetic differentiation of populations (23).

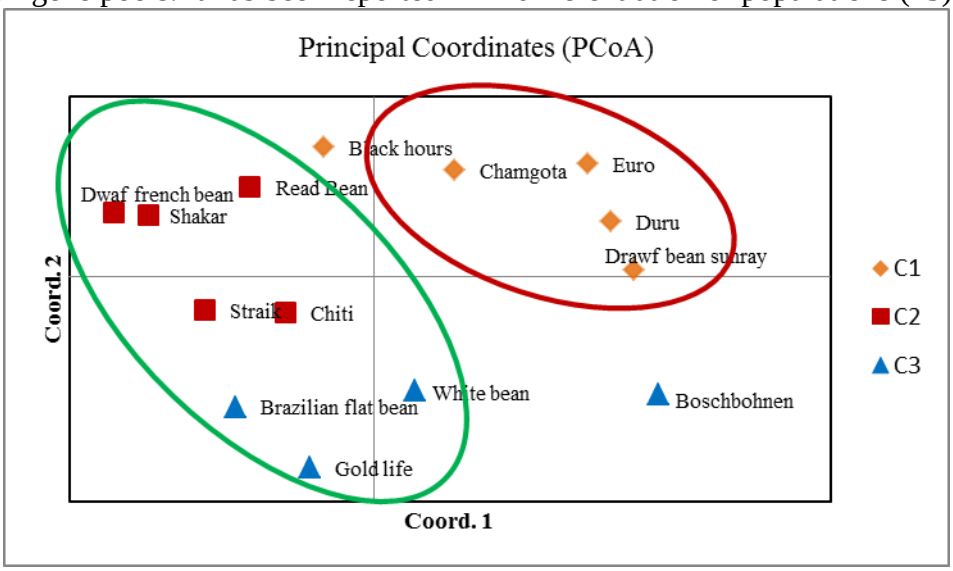


**Figure 4. Principal coordinate analysis (PCoA) of 14 common bean genotypes based on the data of 26 SSR markers**

**Analysis of molecular variance**

Analysis of Molecular variance (AMOVA) was conducted based on SSR alleles variability to find out the differences among the population (Table 6). The highest percentage of the variation was attributable more to differences among individuals within populations (47%). While the variation among populations (25%) and within individuals were 25% and 28%, respectively. Blair, et al. (10) observed similar results of 20.3% of variation among five populations. In contrast De Luca, et al. (16) found that 59.83% of the variation was among their studied populations. The percentage of variation obtained from AMOVA is different from one investigation to another based on geographical origins, polymorphism and genetic diversity in the studied materials. The level of genetic differentiation was classified based on (fixation index=$F_{ST}$) values into: low differentiation ($F_{ST}$ = 0.00–0.05), moderate differentiation ($F_{ST}$ =0.05–0.15) and a high level of differentiation ($F_{ST}$ of >0.30). A high level of genetic differentiation value of 0.250 was observed at a significant level (P-value = 0.001) between all the populations (29). Similarly, in other studies using common bean and SSR markers, a very high genetic differentiation $F_{ST}$ of 0.450, 0.456, 0.665 was reported by Carvalho, et al. (13), Gioia, et al. (23) and Vidak, et al. (54), respectively. Finally, the gene flow (*Nm*) value was 0.749 indicating intermediate levels of gene exchange between sub-populations according to Slatkin (49).

**Table 6. Analyses of molecular variance for the studied common bean**

| Source | Df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| **Among Pops** | **2** | **54.468** | **27.234** | **1.925** | **25%** |
| **Among Individuals within populations** | **11** | **102.925** | **9.357** | **3.589** | **47%** |
| **Within Individuals** | **14** | **30.500** | **2.179** | **2.179** | **28%** |
| **Total** | **27** | **187.893** | | **7.693** | **100%** |
| **F st** | **0.250** | **(p < 0.001)** | | | |
| **Nm** | **0.749** | | | | |

Clustering the genotypes based on morphological traits and DNA marker data are not fully matched, however some of the genotypes are persistent to group together; such as Dwarf French bean with Shakar; Boschbohnen with white bean; and Duru with Euro and Balack horse. The distortion of other genotypes clustering for both data set could be due to high influencing of morphological markers by environmental variances, that would reduce their selection efficiency in the field, especially for the quantitative traits (38).

Small population size of the genotypes and unsatisfaction number of DNA marker to cover the entire genome could be another reason of this distortion (57). The genotyping analysis has distributed the genotypes into three clusters, regardless of their geographical distributions. Md-23 primer had the highest value of gene diversity and PIC. The current results revealed that SSR markers could be successfully employed for the amplification of genotypes of common bean. Cluster analysis (STRUCTURE, UPGMA and PCoA) results revealed the presence of two separated subgroups of Andean and Mesoamerican origin. The results of phenotypic and molecular genetic structure analysis in present study will shorten the path for the researchers to make an informative selection for the further improvement program of common beans in the region.

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