Genetic and molecular analysis of seed coats and pollen grains of *Phaseolus vulgaris* L. during their developmental stages

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ABSTRACT

Phaseolus vulgaris L. (common bean) is a valuable and highly nutritious food legume which exhibits a wide variety of seed coat patterns and colors. Such colors are controlled by a group of specific genes that regulate the flavonol and anthocyanin biosynthetic pathways. The current study aims to describe genetic and molecular analysis of three seed types of *P. vulgaris* var. including strike, contender, and wonder types that possess different seed coats colors (creamy, brown to deep brown, and reddish spotty (pinto) beans. We assessed the phenotypic characteristics of the seeds and seed coats based on seed germination and seedling growth parameters from one hand and macro, micro-phenotypic aspects of seed coat using stereomicroscope and scanning electron microscope (SEM) from other hand. The results indicated that all markers used from phenotypic to DNA-based molecular markers differ in their resolving power to detect genetic variations, identification, genetic structure, and type of data they generate for each species. Phenotypical and protein-based markers were dependent on the gene expression of DNA at exons (coding) region only. On the other hand, the DNA-based markers were dependent on coding (exons) and non-coding (intron) regions and are not governed by above external factors. In conclusion, the DNA-based markers seemed to be the best-suited molecular assay for fingerprinting and assessing genetic structure of each one of *P. vulgaris* L. variety with high accuracy. Also, *P. vulgaris* var. with brown to deep brown seed coat color was more pronounced than the other two varieties.

Keywords: Genetic; Molecular analysis; Seed coats; Pollen grains; *Phaseolus vulgaris* L.

INTRODUCTION

Seeds of *Phaseolus vulgaris* L. (common bean) show a very large variation in size, shape, and color. This fact is quite obvious to anyone who has grown beans in the garden, used them as a research model, or visited produce markets in Africa, and South America. The variation in seed phenotype of bean is an important to commerce as it is interesting to the scientist and naturalist. The scientist view color variation in bean as an opportunity to codify the responsible genes and determine their biochemical functions. *Phaseolus vulgaris* L. exhibits a wide variety of seed coat patterns and colors. From a historical perspective, extensive genetic analyses have identified specific genes that control seed coat pattern (McClean *et al.,* 2002).

In flowering plants, pollen grain is the male gametophyte. Upon pollination, it germinates and develops into a fast-growing cytoplasmic extension, the pollen tube, which ultimately delivers the sperm into the ovary. Studies on pollen development and pollen-tube growth have their origins and focused on the role of the male gametophyte in sexual plant reproduction. The biological relevance of its role, and the uniqueness of this kind of cellular organization, have made pollen as one of the best-known models in plant cell biology. In this respect McCormick (2004) and Boavida *et al*. (2005) showed the key biological role of pollens. In contrast, the genetic background of its development is not well characterized. While, Becker and Feijó (2007) investigated some genes involved in the development of pollens and proved pollen stands as an excellent target for molecular-biology-based The current study aims to describe genetic and molecular analysis of three seed types of *P. vulgaris* var. including strike, contender, and wonder types that possess different seed coats colors (creamy, brown to deep brown, and reddish spotty) beans.

MATERIALS AND METHODS

Chemical materials

All chemicals used in the present study were obtained from King Saud University store, Dar Al Zahrawi Medical LLC and Salehiya Trading E.S.T.

Plant material

Three commercial varieties of *Phaseolus vulgaris* L. (common bean) were used in this study. These varieties were *P. vulgaris* L. cv. strike, contender, and wonder. Their name and origin are showed in Table (1).

Phenotypic parameters

Determination of seed germination parameters of P. vulgaris L. varieties

20 surface sterilized *P. vulgaris* L. seeds of each variety were placed at the same time on sterilized cotton wool, saturated with distilled water, and placed in sterilized Petri dishes in triplicate. The Petri dishes were placed in a plant growth chamber with a light and dark cycle at 25 °C to determine seed germination parameters. Number of germinated seeds for each repetition was recorded after the radical reached 2 mm long. The emergence radical was taken as index of seed germination after 2, 4, and 6 days. Germination parameters were the final germination percent (FGP) as per ISTA, (1985); and mean daily germination (MDG) which is an index of

daily germination rate (Scott et al., 1984). Also, means time of germination (MTG), an index for germination rate was evaluated as described by Ellis and Roberts (1981); and coefficient of velocity of germination (CVG), an index for germination speed was estimated according to Maguire (1962). These parameters were evaluated by count as below:

The final germination percent (FGP):

FGP = number of germinated (2,4,6) daus/number of seeds × 100.

Means daily germination (MDG):

MDG = FGP \D

Means

MTG = ∑ (NI × DI)\N

Where n is the number of seeds germinated at day i, d the incubation period in days and N the total number of seeds germinated in the treatment.

Coefficient of velocity of germination (CVG):

CVG = no. of germinated seeds on day 1\1+no. of germinated seeds on day n.\n

n referred to number of days after sowing

Plantation of germinated seeds of each P. vulgaris L. varieties

Triplicate of *P. vulgaris* germinated seeds were sown in plastic basins had the same size (length 70 × width 40 cm) inside glass greenhouses at the Department of Botany and Microbiology, College of Science, KSU. At seedling stage (15 days) and vegetative stage (30 days), phenotypic seedling growth parameters were recorded.

Determination of seedling growth parameters of P. vulgaris

At the seedling stage and the vegetative stage, mean lengths (cm) of root, shoot, and seedlings, mean root/ shoot ratios, leaf parameters represented in number of leaves per plant and leaf surface area (cm^2), seedling dry weight (g) were measured in addition to seedling vigour index (VI) I and II was computed based on Eq. (1 and 2):

(VI) I = Seedling lengths (cm) × Germination (%) Eq 1

(VI) II= Seedling weight (g) × Germination (%) Eq 2

DNA-marker analysis using RAPD-PCR

Isolation of genomic DNA

A genomic DNA was extracted from *P. vulgaris* L. seed using the hexadecyltrimethyl ammonium bromide method as described by Kit and Chandran (2010).

Qualitative and quantitative analyses of extracted DNA

DNA yield was measured using a UV-visible spectrophotometer (PerkinElmer, Waltham, MA, USA) at 260 nm. DNA purity was determined by calculating the absorbance ratio at A260/280 nm. Polysaccharide contamination was assessed by calculating the absorbance ratio at A260/230 nm (Wilson and Walker, 2005). For quality and yield assessments, electrophoresis test was performed for all DNA samples on 0.8 % agarose gels that were stained with ethidium bromide; the bands were observed using a gel documentation system (AlphaInnotech, San Leandro, CA, USA) and compared with a known standard lambda DNA marker sample.

PCR amplification using random primers of RAPD

The PCR reaction mixture contained 2.5 µL 10 x buffer with 15 mM $MgCl_2$ (Fermentas, Vinius, Lithuania), with 0.25 mM of each dNTP (Sigma, St. Louis, MO, USA), 0.3 µM primer, 0.5 U *Taq* DNA polymerase (Sigma), and 50 ng template DNA. The PCR reaction was performed in a Palm Cycler apparatus (Corbett Research) using the following method: initial denaturation of 4 min at 95 °C followed by 40 cycles of 1 min at 95 °C, 1 min at 38 °C, and 2 min at 72 °C with final extension at 72 °C for 10 min and a hold temperature of 4 °C. A total of 20 random DNA oligonucleotide primers (10-mer) were independently used in the PCR reactions (University of British Columbia, Canada) according to Williams *et al.* (1990) with some modifications. Only 7 primers (P-01, 02, 03, 04, 05, 06 and 07) successfully generated reproducible DNA amplification products. For DNA amplification, the PCR was run for 35 cycles, which consisted of a denaturation step (1 min at 95 °C), annealing step (1 min at 35 °C), and elongation step (2 min at 72 °C). After 34 cycles, a final extension period was added (5 min at 72 °C). Amplification products were electrophoresed on 1.5 % agarose gel (Sigma) in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA, pH 8). The gel was run at 100 V constant voltage for 1 h. Gels were stained with 0.2 µg /mL ethidium bromide for 15 min. The PCR products were visualized under a UV light transilluminator. The 100-base pair DNA ladder (Gibco-BRL, Grand Island, NY, USA) was loaded into the first lane of each gel to evaluate band sizes. The gels were photographed under UV light using a gel documentation system (Bio-Rad, Hercules, CA, USA).

Scoring and data analyses

After separating PCR products by agarose gel electrophoresis, the gels were visualized using a Photo Print (VilberLourmat, France) imaging system. Quantitative variations in band numbers and band sizes as well as band intensity were analyzed using Bio-One D++ software (VilberLourmat, France). Data were scored as the presence or absence of DNA bands.

Statistical analyses

Each experiment for seed germination parameters and seedling growth parameters was carried out in t replicates. Data are expressed as means ± standard deviation (SD). The obtained data were statistically analyzed by using One Way ANOVA. Probability level was tested at 0.05.

RESULTS

Phenotypic parameters based on seed germination and seedling growth

Results of seed germination parameters

Estimation of phenotypic parameters of germinating seeds of three *P. vulgaris* L. varieties depended on the final germination percent (FGP), means daily germination (MDG), Means time of germination as germination rate (MTG), and coefficient of velocity of germination (CVG) as germination speed as depicted in Table (2) and Figure (1). Seeds were considered germinated when they exhibited a radical extension of >2 mm. Counts of germinated seeds were made for 2, 4, and 6 days to determine seed germination parameters. The obtained results showed that all seed germination parameters (FGP, MDG, MTG, and CVG) reached maximum values at *P. vulgaris* L. var. contender. FGP increased with increasing germination period while MDG decreased. At this variety, the maximum values of FGP reached (100%) at the time period 6 days, while the maximum values of MDG values (30%) at the time period 2 days compared to their values at the other two varieties that reached (30% and 5%) at the same time period. On the other hand, MTG and CVG values reached 10.80 % and 20.75 % respectively at *P. vulgaris* L. var. contender compared to their values at the other two varieties that reached 9.33 % and 20.75 % respectively. Estimation of Phenotypic parameters of germinating seeds of three *P. vulgaris* L. varieties based on the final germination percent (FGP); means daily germination (MDG); Means time of germination as germination rate (MTG); and coefficient of velocity of germination (CVG) as germination speed are listed in Table (2) and Figure (1). Seeds were considered germinated when they exhibited a radical extension of >2 mm. Counts of germinated seeds were made for 2, 4, and 6 days to determine seed germination parameters. The results obtained showed that all seed germination parameters (FGP, MDG, MTG, and CVG) reached maximum values at *P. vulgaris* L. var. contender. FGP increased with increasing germination period while MDG decreased. At this variety, the maximum values of FGP reached (100%) at the time period 6 days while the maximum values of MDG values (30%) at the time period 2 days compared to their values at the two other varieties which reached (30% and 5%) at the same time period. On the other hand, MTG, and CVG values reached 10.80% and 20.75% respectively at *P. vulgaris* L. var. contender compared to their values at the two other varieties that reached 9.33% and 20.75% respectively.

Results of seedling growth parameters

The results presented in Table (3) and Figure (2) show the seedling growth parameters of three *P. vulgaris* L. varieties after (15 and 30 days from planting of seeds) depended on mean lengths (cm) of root (shoot, and seedlings), mean root/ shoot ratios, leaf parameters represented in number of leaves per plant and leaf surface area (cm^2), seedling dry weight (g), in addition to seedling vigour index (VI) I and II. The current data showed significant increasing in all seedling growth parameters of *P. vulgaris* L. var. contender more than the strike and wonder varieties. On the other hand, the wonder variety showed slightly increase in these parameters than the strike variety. The maximum values of seedling growth parameters after 30 days showed at *P. vulgaris* L. var. contender were 25.36±2.56 cm, 15.43±1.10 cm, and 40.76±2.00 cm for mean lengths of root, shoot, and seedlings respectively compared to the minimum values of these parameters at the strike variety which recorded 12.00±1.32, 10.83±0.72, and 22.83±2.03 cm respectively.

The results presented in Table (3), show the seedling growth parameters of three *P. vulgaris* L. varieties after (15 and 30 days from planting of seeds) based on mean lengths (cm) of root, shoot, and seedlings; mean root/ shoot ratios, leaf parameters represented in number of leaves per plant and leaf surface area (cm^2), seedling dry weight (g) in addition to seedling vigour index (VI) I and II. The current data showed significant increasing in all seedling growth parameters mentioned above at *P. vulgaris* L. var. contender more than the strike and wonder varieties. On the other hand, the wonder variety showed slightly increased in these parameters than the strike variety. The maximum values of seedling growth parameters after 30 days showed at *P. vulgaris* L. var. contender were 25.36±2.56 cm, 15.43±1.10 cm, and 40.76±2.00 cm for mean lengths of root, shoot, and seedlings respectively compared to the minimum values of these parameters at the strike variety which recorded 12.00±1.32 cm, 10.83±0.72, and 22.83±2.03 cm respectively.

Seed viability or seedling vigour index are the set of characteristic that determine the activity and behaviour of the seed germination under the environmental factors in different geographical regions. Seedling vigour was computed based on the vigour index (VI) I of seedling length and vigour index (VI) II of seedling dry weight varied between

the three varieties. contender variety showed significant increasing over two other varieties in vigor indices which reached the maximum values of (4523.00 and 493.00) for (VI) I and (VI) II respectively compared to vigor indices values recorded at strike variety which reached minimum values (565.99 and 58.38) respectively.

On the other hand, number of leaves per plant was the same at the three varieties (3 leaves) while significant variation in respect of leaf surface among three varieties was found. The maximum number of leaf surface area after 30 days was produced by contender variety and reached the values of 63.16±1.78 cm^2. The corres ponding minimum number of leaf surface area was recorded by strike variety reached the value of 23.71±4.72 cm^2.

Phenotypic features of flower color and fruiting criteria

The results presented in Table (4), Figures (3 and 4-a,b) show variations in the flower color and fruiting criteria of three *P. vulgaris* L. varieties based on the mean number of pods/plant, the mean number of seeds/pod, average weight of 100seeds/g, and Seed sizes measured by weight. The flower color ranged between white to yellowish for strike variety, Deep Violet for contender variety, and yellowish to pale Violet for wonder variety. On the other hand, contender variety showed increasing in number of green pods/plant and number of green seeds / pod than the two other varieties. The average weight of 100seeds/gm showed clear variations among varieties. The maximum weight (60.00 ± 0.40 g) was obtained in contender variety while minimum weight (20.80 ± 0.40 g) recorded in strike variety. Moreover, seed sizes according to weight showed variations among varieties ranged from small sizes in strike variety and large sizes in contender and wonder varieties.

Macro- and micro-phenotypical aspects of *P. Vulgaris* L. varieties

Macro-phenotypical aspects

Macro-phenotypical aspects of three *P. vulgaris* L. varieties using Stereomicroscopy model are given in Table (5), and Figure (5). Data obtained by stereomicroscope investigation showed 8 criteria based on the seed characters including, shape, texture, phenotypic seed color, average seed sizes by length × width (L × W) mm, Position, and hilum sizes by length x width (L × W) mm. The present data observed that each variety had specific macro-phenotypical aspects as follows:

A- Phaseolus vulgaris var. strike

The seed shape was kidney, with glabrous striped texture, and phenotypic creamy color. Seed dimensions (L × W) were (12.83± 0.43 × 5.85± 0.52 mm) in long. Average seed size was 75.05± 0.48 mm. Position and shape of hilum were central with laterally appendages and without outgrowth at the top, while hilum size was 3.08×0.35=0.9 mm.

B- Phaseolus vulgaris var. contender

The seed shape was cylinder to oblong, with glabrous texture, and brownish to deep brown phenotypic color. Seed dimensions (L × W) were (16.58±0.23 × 8.65±0.40mm) in long and average seed size was 143.42± 0.52 mm. Position and shape of hilum were central laterally extended with one out growth at the top, while hilum size was 3.87 × 0.93=3.60 mm.

C- Phaseolus vulgaris var. wonder

The seed shape was broadly oblong-ellipsoid, with glabrous spotty texture, and reddish spotty (pinto) phenotypic color. Seed dimensions (L × W) were (13.12±0.63 × 8.07±0.13mm) in long and average seed size was 105.89± 0.20mm. Position and shape of hilum were subapical elliptical with two elevated out growths at the top while hilum size was 3.37×0.40=1.92mm.

Molecular analysis

DNA-marker analysis using RAPD-PCR

Estimation of genetic variation is very important for crop improvement programs. In the current study, we examined the level of genetic variation among colored seeds of three *P. vulgaris* L. varieties using RAPD analysis. Twenty random primers were used for the RAPD analysis, in which only seven primers of them (P-1, 2, 3, 4, 5, 6 and 7) succeeded to produce clear reproducible DNA bands and gave satisfactory results with many alterations in the RAPD profiles. The code and sequences of the seven RAPD primers listed in Tables (6-13).

Survey of RAPD polymorphisms among dry seeds of three P. vulgaris L. varieties using RAPD-PCR technique.

The DNA banding patterns revealed by RAPD-PCR used to detect the genetic variations among dry seeds of three *P. vulgaris* L. varieties during seven developmental stages remembered above showed in Table (6) and Figures (6-8). RAPD revealed that 103 amplified DNA products had highly polymorphism value (100%) based on band numbers, with sizes ranging from 324.96-959.76 base pairs, and band intensity. An average of 14.71 bands per primer was scored. Out of which, 93 bands were polymorphic with value 90.29% (83 bands were unique, 10 bands were non- unique with values 80.58% and 9.71% respectively).

The maximum number of amplified DNA products (36 bands) was recorded at strike variety

with value of 34.95%. The minimum number of amplified DNA products (33 bands) was found in wonder variety with value of 32.04 %. On the other hand, Primer-07 produced the maximum number of amplified DNA products 24 with value 23.30 % and unique bands, 22 with a value of 91.67 %, while Primer-2 produced the minimum number of amplified DNA products 8 with value 7.77 % and unique bands, 6 with a value of 75.00 %

Survey of RAPD polymorphisms among dry seed coats of three P. vulgaris L. varieties using RAPD - PCR technique

The DNA banding patterns revealed by RAPD-PCR used to detect the genetic variations among dry seed coats of three *P. vulgaris* L. varieties during seven developmental stages remembered above showed in Table (7) and Figures (6-13). RAPD revealed that 104 amplified DNA products had highly polymorphism value (100%) based on band numbers, with sizes ranging from 335.51-951.23 base pairs, and band intensity. An average of 14.86 bands per primer was scored. Out of which, 94 bands were polymorphic with value 90.38 % (84 bands were unique, 10 bands were non- unique with values 80.76 % and 9.62 % respectively).

The maximum number of amplified DNA products (35 bands) recorded at strike and contender varieties with value of 33.65 %. The minimum number of amplified DNA products (34 bands) found at wonder variety with value of 32.69 %. Primer - 7 produced the maximum number of amplified DNA products 24 with value 23.10 % while Primer - 2 produced the minimum number 6 with value 5.76 % On the other hand, Primer - 3 produced the maximum number unique bands, 24 with a value of 100 % while Primer – 2 produced the minimum number of unique bands, 6 with a value of 100 %.

Survey of RAPD polymorphisms among imbibed seed coats of three P. vulgaris L. varieties using RAPD–PCR technique.

The DNA banding patterns revealed by RAPD - PCR used to detect the genetic variations among imbibed seed coats of three *P. vulgaris* L. varieties during seven developmental stages remembered above showed in Table (8) and Figures (6-13). RAPD revealed that 106 amplified DNA products had highly polymorphism value (100 %) based on band numbers, with sizes ranging from 330.19 - 950.41 base pairs, and band intensity. An average of 15.14 bands per primer was scored. Out of which, 98 bands were polymorphic with value 92.45 % (90 bands were unique, 8 bands were non - unique with values 84.91 % and 7.54 % respectively).

The maximum number of amplified DNA products (36 bands) recorded at strike variety with value of 33.96 %. The minimum number of amplified DNA products (35 bands) found in contender and wonder varieties with value of 33.20 %. Primer - 7 produced the maximum number of amplified DNA products 24 with value 22.64 % while Primer - 2 produced the minimum number 6 with value 5.66 %. On the other hand, Primer -7 produced the maximum number unique bands, 24 with a value of 100 % while Primer –2 produced the minimum number of unique bands, 4 with a value of 66.67 %.

Survey of RAPD polymorphisms among dry embryos of three P. vulgaris L. varieties using RAPD–PCR technique

The DNA banding patterns revealed by RAPD-PCR used to detect the genetic variations among dry embryos of three *P. vulgaris* L. varieties during seven developmental stages remembered above showed in Table (9) and Figures (6-13). RAPD revealed that 100 amplified DNA products had highly polymorphism value (100 %) based on band numbers, with sizes ranging from 304.83- 948.68 base pairs and band intensity. An average of 14.29 bands per primer was scored. Out of which, 92 bands were polymorphic with value 92.00 % (84 bands were unique, 8 bands were non- unique with values 85.00 % and 8.00 % respectively).

The maximum number of amplified DNA products (35 bands) recorded at contender variety with value of 35.00 %. The minimum number of amplified DNA products (32 bands) found in strike variety with value of 32.00 %. Primer – 7 produced the maximum number of amplified DNA products 24 with value 24.00 % while Primer - 2 produced the minimum number 6 with value 6.00 %. On the other hand, Primer - 7 produced the maximum number unique bands, 24 with a value of 100 % while Primer – 2 produced the minimum number of unique bands, 4 with a value of 66.66 %.

Survey of RAPD polymorphisms among imbibed embryos of three P. vulgaris L. varieties using RAPD-PCR technique:

The DNA banding patterns revealed by RAPD-PCR used to detect the genetic variations among imbibed embryos of three *P. vulgaris* L. varieties during seven developmental stages remembered above showed in Table (10) and Figures (6-13). RAPD revealed that 102 amplified DNA products had highly polymorphism value (100 %) based on band numbers, with sizes ranging from 330.19-965.49 base pairs and band intensity. An average of 14.57 bands per primer was scored. Out of which, 94 bands were polymorphic with value 92.2 % (86 bands were unique, 8 bands were non- unique with values 84.31 % and 7.84 % respectively).

The maximum number of amplified DNA products (35 bands) recorded at contender variety

with value of 34.31 %. The minimum number of amplified DNA products (33 bands) found at wonder variety with value of 32.35 %. Primer - 7 produced the maximum number of amplified DNA products 25 bands with value 24.51 % while Primer - 2 produced the minimum number 6 with value 5.88 %. On the other hand, Primer - 7 produced the maximum number unique bands, 23 with a value of 92.00 % while Primer - 2 produced the minimum number of unique bands, 6 with a value of 100 %.

Survey of RAPD polymorphisms among radicals of three P. vulgaris L. varieties using RAPD-PCR technique

The DNA banding patterns revealed by RAPD-PCR used to detect the genetic variations among radicals of three P. vulgaris L. varieties during 7 developmental stages are shown in Table (11). RAPD revealed that 108 amplified DNA products had highly polymorphism value (100 %) based on band numbers, with sizes ranging from 335.51 - 961.47 base pairs and band intensity. An average of 15.43 bands per primer was scored. Out of which, 101 bands were polymorphic with value 93.52 % (94 bands were unique, 7 bands were non- unique with values 87.04 % and 6.48 % respectively). The maximum number of amplified DNA products (38 bands) recorded at wonder variety with value of 35.19 %. The minimum number of amplified DNA products (35 bands) found at strike and contender varieties with value of 32.41 %. Primer - 7 produced the maximum number of amplified DNA products 30 bands with value 27.78 % while Primer - 2 produced the minimum number 6 with value 5.56 %. On the other hand, Primer - 7 produced the maximum number unique bands, 22 with a value of 73.33 % while Primer – 2 produced the minimum number of unique bands, 6 with a value of 100 %.

Survey of RAPD polymorphisms among pollen grains of three P. vulgaris L. varieties using RAPD-PCR technique

The DNA banding patterns revealed by RAPD-PCR used to detect the genetic variations among pollen grains of three *P. vulgaris* L. varieties during 7 developmental stages remembered above showed in Table (12) and Figures. (6-13). RAPD revealed that 107 amplified DNA products had highly polymorphism value (100 %) based on band numbers, with sizes ranging from 387.42 - 961.47 base pairs, and band intensity. An average of 15.29 bands per primer was scored. Out of which, 101 bands were polymorphic with value 94.39 % (95 bands were unique; 6 bands were non- unique with values 88.78 % and 5.61 % respectively).

The maximum number of amplified DNA products (37 bands) recorded at contender variety with value of 34.57 %. The minimum number of amplified DNA products (35 bands) found in strike and wonder varieties with value of 32.71 %. Primer - 7 produced the maximum number of amplified DNA products 24 bands with value 22.64 % while Primer – 2 produced the minimum number 7 with value 6.54 %. On the other hand, Primer - 7 produced the maximum number unique bands, 24 with a value of 100 % while Primer -2 produced the minimum number of unique bands, 6 with a value of 100 %.

Comparative amplified DNA banding pattern between each variety at different seed developmental stages

The present study observed that the three varieties of *P. vulgaris* L. had amplified DNA-banding variations during different seed developmental stages based on the number of amplified DNA products and their intensity and amplicon lengths (bp) as listed in Table (13).

Phaseolus vulgaris var. strike

Amplified DNA products ranged from maximum numbers to minimum number which reached 33 DNA bands at the developmental stages (imbibed seed coat and pollen grains) to 25 bands at the developmental stages (dry embryo) respectively.

Phaseolus vulgaris var.contender

Amplified DNA products ranged from mximum numbers to minimum number that reached 32 DNA bands at the developmental stages (Full dry seeds) to 25 bands at the developmental stages (pollen grains) respectively.

Phaseolus vulgaris var.wonder

Amplified DNA products ranged from maximum numbers to minimum number which reached 32 DNA bands at the developmental stages (radicals) to 27 bands at the developmental stages (dry embryo) respectively.

The Amplified DNA products (unique bands) generated by RAPD-PCR for identifying the three P. vulgaris L. varieties during different seed developmental stages

The unique bands are specifying for one variety without the others and can use as markers for amplified DNA products alterations among these varieties. The resulting amplified DNA profiles showed different patterns, indicating variability among varieties collected from different ecogeographical origins and different seed developmental stages. The number of amplified DNA unique bands and their amplicon sizes (bp) listed in Table (13) and Figure (13).

Full dry seeds developmental stage

Amplified DNA products generated maximum number of unique bands (31 bands) recorded at strike variety while minimum number of unique bands (25 bands) recorded at contender variety, each

group of these unique bands recorded different amplicon sizes (bp).

Developmental stage of dry seed coats.

Amplified DNA products generated maximum number of unique bands (31 bands) recorded at wonder variety while minimum number of unique bands (26 bands) recorded at contender variety, each group of these unique bands recorded different amplicon sizes (bp).

Developmental stage of imbibed seed coats

Amplified DNA products generated maximum number of unique bands (33 bands) recorded at strike variety while minimum number of unique bands (28 bands) recorded at contender variety, each group of these unique bands recorded different amplicon sizes (bp).

Developmental stage of dry embryo.

Amplified DNA products generated maximum number of unique bands (31 bands) recorded at wonder variety while minimum number of unique bands (25 bands) recorded at strike variety, each group of these unique bands recorded different amplicon sizes (bp).

Developmental stage of imbibed embryo

Amplified DNA products generated maximum number of unique bands (30 bands) recorded at strike variety while minimum number of unique bands (28 bands) recorded at contender and wonder varieties, each group of these unique bands recorded different amplicon sizes (bp).

Developmental stage of radicals.

Amplified DNA products generated maximum number of unique bands (32 bands) recorded at strike and wonder varieties while minimum number of unique bands (30 bands) recorded at contender strike and wonder variety, each group of these unique bands recorded different amplicon sizes (bp).

Developmental stage of pollen grains.

Amplified DNA products generated maximum number of unique bands (33 bands) recorded at strike variety while minimum number of unique bands (30 bands) recorded at and wonder strike and wonder variety, each group of these unique bands recorded different amplicon sizes (bp).

DISCUSSION

Phenotypic parameters have based on germinating seeds and seedling growth of three *P. vulgaris* L. varieties

The phenotypic analysis platforms described here, in conjunction with metabolic and gene expression profiling analyses using protein based-biochemical and DNA based- molecular markers conducted in parallel provide a robust method for the high throughput functional analysis of plant genes. Seed germination and seedling establishment are the two most important events in the life cycle of plants. It is important to know that, understanding the sources of phenotypic variation in organisms is central to understand the genetic variation induced in plants and the responses of these plants to their environment.

DNA-marker analysis using RAPD-PCR

In the current study, RAPD analysis revealed distinctive qualitative and quantitative variations among the three *P. vulgaris* L. varieties at seed developmental stages separately in the RAPD banding patterns based on the number of gene products, amplified DNA sizes, band intensities, and appearance or disappearance of DNA bands, leading to high levels of DNA polymorphisms. The number of amplified DNA products generated by seven primers used in present study may be related to change in environmental factors in their ecogeographic regions that leading to change in the number and direction of genome sequences complementary to the primer, template quantity and less number of annealing sites in the genome as confirmed by Kernodle *et al.,* (1993). High levels of polymorphism generated by RAPD are considered a better parameter for measuring genetic variation patterns and reflect heritable changes in the nucleotide sequences, both in coding and non-coding regions (Lal *et al.,* 2011). These DNA polymorphisms may result from DNA structural changes within base-pair sequences of DNA between oligonucleotide primer binding sites in the genome during DNA replication or gene expression under genotoxic environmental stresses, such as a nucleotide substitution within a target site, an insertion or deletion of a DNA-fragment within the amplified regions, rearrangements of genomic DNA, or inversions, translocations, and transpositions of genes (Welsh and McClelland, 1991). DNA polymorphisms among different three *P. vulgaris* L. varieties may have resulted from the interaction of DNA with ROS such as hydroxyl radical (•OH) generated by abiotic oxidative stress levels at ecogeographical regions, leading to oxidative DNA damage at both the purine and pyrimidine bases, the deoxyribose backbone, change in gene expression, and abnormal oxidative proteins (Sanghera *et al.*, 2013). These changes are exhibited as the loss or deletion of DNA bands (polymorphic) that may be very valuable for DNA fingerprinting and examining genetic variations among different varieties or insertions of new amplified DNA bands (unique) or changes of nitrogenous base or changes

in fragment size, and consequently different DNA lengths with highly polymorphism levels.

On the other hand, the appearance of new DNA band (unique bands) occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change or because some changes in DNA sequence have occurred due to mutations, large deletions, and/or homologous recombination. Appearance of new bands may also be the results of genomic template instability related to the level of DNA damage, the efficiency of DNA repair and replication (Atienzar *et al.*, 1999). As suggested by Liu *et al.* (2005), modifications of band intensity and lost bands are likely to be due to one or a combination of the following events: (1) changes in oligonucleotide priming sites due mainly to genomic rearrangements and less likely to point mutations; (2) DNA damage in the primer binding sites; (3) interactions of DNA polymerase in test organism with damaged DNA. RAPD analyses in conjunction with other biomarkers such as phenotypic parameter, biochemical protein-based, etc., could prove to be a powerful genetic variation tool for different *P. vulgaris* L. varieties.

CONCLUSION

In conclusion, the contender bean seeds with brown to deep brown seed coat color were more pronounced than the other two varieties as they recorded significant stimulations and highest values in all seed germination and seedling growth parameters supporting by their vigour of seedling lengths and dry seedling weights as indicator. In addition, they recorded the pronounced flower color and fruiting criteria values higher than the other varieties. The RAPD analysis could be successfully used for the estimation of genetic variability among germplasms of different *P. vulgaris* L. varieties. The effective and the credibility of this technique were used in gene banks for identification and differentiation of *P. vulgaris* L. varieties.

REFERENCES

Atienzar, F.A., Conradi, M., Evenden, A.J., Jha, A.N., Depledge, M.H., 1999. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna to benzo[a]pyrene. Environ. Toxicol. Chem., 18, 2275-2282.

Becker, J.D., Feijó, J.A., 2007. How Many Genes are needed to make a Pollen Tube? Lessons from Transcriptomics. Ann. Bot., 100 (6), 1117-1123.

Boavida, L.C., Becker, J.D., Feijo, J.A., 2005. The making of gametes in higher plants. Int. J. Dev. Biol., 49 (a), 595-614.

Ellis, R.A., Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol. 9, 373-409.

ISTA, 1985. International seed testing association. international rules for seed testing. Seed Sci. Technol., 13, 299-335.

Kernodle, S.P., Cannon, R.E., Scandalios, J.G., 1993. Concentration of primer and template qualitatively affects product in RAPD-PCR. Biotechniques. 1, 362-364.

Kit, Y.S., Chandran S.A., 2010. Simple, rapid and efficient method of isolating DNA from Chokanan mango (*Mangifera indica* L.) Afr. J .Biotechnol., 9 (36), 5805–5808.

Lal, S., Mistry, K.N., Shah, S.D., Thaker, R., 2011. Genetic diversity assessment in nine cultivars of *Catharanthus roseus* from Central Gujarat (India) through RAPD, ISSR and SSR markers. J. Res. Biol., 1, 667-675.

Liu, W., Li, P.J., Qi, X.M., Zhou, Q.X, Zheng, L., Sun, T.H., Yang, Y.S., 2005. DNA changes in barley (Hordeum vulgare) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere. 61, 158–167.

Ma, H., 2005. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. Ann. Rev. Plant Biol., 56, 393-434.

Maguire, J.D., 1962. Seed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2, 176-177.

McClean, P.E., Lee, R.K., Otto, C., Gepts, P., Bassett, M.J., 2002. Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). J. Heredity, 93 (2), 148-152.

McCormick, S., 2004. Control of male gametophyte development. Plant Cell, 16 (Suppl.), 142-153.

Sanghera, G.S., Malhotra, P.K., Sidhu, G.S., Sharma, V.K., 2013. Genetic engineering of crop plants for enhanced antioxidants activity. Int. J. Adv. Res. Tech. 2, 428-458.

Scott, S.J., Jones, R.A., Willams, W.A., 1984. Review of data analysis methods for seed germination. J. Crop Sci., 24, 1192-1199.

Welsh, J., McClelland, M., 1991. Genomic fingerprinting using arbitrarily primed PCR and a matrix of pairwise combinations of primers. Nucleic Acids Res., 19, 5275-5279.

Williams, J.G.K., Kubelik, AR., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18, 6531–6535.

Wilson, K., Walker, J., 2005. Principles and Techniques of Biochemistry and Molecular Biology, 7th (Eds.), Cambridge Textbooks, p. 759.

Table 1. the name and origin of *P. vulgaris* L. varieties.

Code	*P. vulgaris* var	Origin
A	Super Strike	USA
B	Early Contender Bush Beans	USA
C	Kentucky Wonder Pole Beans	Turkey

Table 2. Seeds germination parameters of *P. vulgaris* L. var; A- strike, B- contender, C- wonder. Values are means ± SD (n=3).

Code	Mean of Seed Germination Parameters ± SD after							
	2 days		4 days		6 days			
	FGP	MDG	FGP	MDG	FGP	MDG	MTG	CVG
A	10±0.00	5±0.00	20±0.00	5±0.00	30±0.00	5±0.00	9.33	4.50
B	60*±0.00	30*±0.00	90*±0.00	22.50*±0.00	100*±0.00	16.66*±0.00	10.80	20.75
C	10±0.00	5±0.00	20±0.00	5±0.00	30±0.00	5±0.00	9.33	4.50
LSD at 5%	0.00	0.00	0.00	0.00	0.00	0.00	-	-

Table 3. Seedling growth parameters of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, values are means ±SD (n=3).

Code	Mean of Seedling growth parameters ±SD after																
	15 days									30 days							
	Mean Lengths (cm)			Mean Root /Shoot Ratios	Leaf parameters		Seedling Dry Weight (g)	(VI) I of Seedling length	(VI) II of Seedling Dry Weight	Mean Lengths(cm)			Mean Root /Shoot ratios	Leaf parameters		Seedling Dry Weight (g)	(VI) I of Seedling length	(VI) II of seedling Dry Weight
	Root	Shoot	Seedling		No. of leaves/plant	Surface area (cm2)				Root	Shoot	Seedling		No. of leaves/plant	Surface area (cm^2)			
A	12.00±1.32	10.83±0.72	22.83±2.03	1.10±0.55	2	9.26±1.41	1.24±0.31	599.85	33.05	12.40±1.73	8.83±0.76	21.23±0.97	1.41±0.32	3	23.71±2.57	2.1±0.39	565.99	58.38
B	25.36*±2.56	15.43*±1.10	40.76*±2.00	1.64*±0.26	2	64.32*±2.42	5.46*±0.38	4076.00	546	27.00*±2.50	18.56*±1.60	45.23*±1.56	1.49*±0.24	3	63.16*±1.78	4.93*±1.05	4523	493
C	14.16±1.25	10.66±1.75	23.5±3.12	1.21±0.25	2	20.63±1.64	2.22±0.19	705.00	66.60	12.70±1.49	8.66±0.28	20.16±3.18	1.35±0.36	3	24.26±1.11	2.71±0.59	603	81.30
LSD at 5%	3.62	2.53	4.86	0.423	0.00	3.71	0.616	-	-	3.89	2.05	4.23	0.61	0.00	3.81	1.46	-	-

Table 4. Phenotypic features of flower color and fruiting criteria *P. vulgaris* L. var; A= strike, B=contender, C= wonder; values are means ±SD (n=3).

Code	Flower color	No. of pods/ plant	No. of seeds /pod	Mean weight of 100 seeds (g)	Seed size by weight **
A	White to yellowish	2.20± 1.90	3.67± 1.30	20.80±0.40	Small , 100 seeds <25 g
B	deep violet	3.00± 1.50	5.67± 1.17	60.00±0.40	Large, 100 seeds > 40 g.
C	yellowish to pale violet	2.66± 1.2	4.67± 1.10	51.40 ±0.05	Large, 100 seeds > 40 g.

Table 5. Macro-phenotypical aspects of seeds of *P. vulgaris* L.var using Stereomicroscopy model. A= strike, B=contender, C= wonder; values are means ±SD (n=3).

				Seed Macro-phenotypical aspects				
				Seed size ±SD			Hilum	
Code	Shape	Texture	Phenotypic seed color	Mean seed Length (mm)	Mean seed Width (mm)	Average seed sizes Length x width (L×W) mm	Position and Shape	Hilum sizes Length x width (L×W) mm
A	Kidney	Glabrous Striped	Creamy	12.83± 0.43	5.85± 0.52	75.05± 0.48	Central with laterally appendages and without outgrowth at the top	3.08×0.35=0.9
B	Cylindered to oblong	Glabrous	Brownish to deep brown	16.58±0.23	8.65±0.40	143.42± 0.52	Central laterally extended with one outgrowth at the top	3.87×0.93=3.60
C	Broadly oblong-ellipsoid	Glabrous spotty	Reddish spotty (pinto)	13.12±0.63	8.07±0.13	105.89± 0.20	Subapical elliptical with two elevated outgrowths at the top	3.37×0.40=1.92

Table 6. RAPD-PCR amplification products of DNA extracted from full dry seeds of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, using seven random primers.

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	952.14-814.37	3	3	3	9	8.74	7	77.78	1	11.11	8	88.89	0	0	100
P-2	TCT GTG CTG G	880.71-815.31	3	3	2	8	7.77	6	75	1	12.5	7	87.5	0	0	100
P-3	GTG ACG TAG G	956.61-766.97	5	6	6	17	16.50	17	100	0	0	17	100	0	0	100
P-4	GGT CCC TGA C	816.41-393.66	5	4	4	13	12.62	13	100	0	0	13	100	0	0	100
P-5	AAT CGG GCT G	880.71-351.99	4	5	5	14	13.59	10	71.42	2	14.28	12	85.72	0	0	100
P-6	AGT CAG CCA C	959.76-788.53	8	5	6	18	17.48	8	44.44	5	27.78	13	72.22	0	0	100
P-7	TGC CGA GCT G	946.15-324.96	8	8	8	24	23.30	22	91.67	1	4.17	23	95.83	0	0	100
Over all total			36	34	33											
						103		8 83	80.5	10	9.71	93	90.29	0	0	100
Total bands in each lane %			34.95	33.01	32.04											

Table 7. RAPD-PCR amplification products of DNA extracted from Dry seed coat of *P. vulgaris* L. var; A-strike, B- contender , and C-wonder using Seven random primers.

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	941.81-814.37	3	3	3	9	8.65	9	100	0	0	9	100	0	0	100
P-2	TCT GTG CTG G	886.70-810.9	2	2	2	6	5.76	6	100	0	0	6	100	0	0	100
P-3	GTG ACG TAG G	946.06-659.02	7	8	9	24	23.1	24	100	0	0	24	100	0	0	100
P-4	GGT CCC TGA C	859.51-363.42	3	5	3	11	10.57	9	81.82	1	9.1	10	90.91	0	0	100
P-5	AAT CGG GCT G	465.39-886.7	3	3	3	9	8.65	9	100	0	0	9	100	0	0	100
P-6	AGT CAG CCA C	951.23-587.27	9	6	6	21	20.2	19	90.47	1	4.76	20	95.23	0	0	100
P-7	TGC CGA GCT G	941.09-335.51	8	8	8	24	23.1	8	33.33	8	33.33	16	66.66	0	0	100
Over all total			35	35	34											
						104		84	80.76	10	9.62	94	90.38	0	0	100
Total bands in each lane %			33.65	33.65	32.69											

Table 8. RAPD-PCR amplification products of DNA extracted from Imbibed seed coat of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, using seven random primers .

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	950.41-808.81	4	4	3	11	10.37	9	81.82	1	9.1	10	90.91	0	0	100
P-2	TCT GTG CTG G	884.3-808.71	2	2	2	6	5.66	4	66.67	1	16.67	5	83.33	0	0	100
P-3	GTG ACG TAG G	949.56-604.03	3	7	7	17	16.1	17	100	0	0	17	100	0	0	100
P-4	GGT CCC TGA C	850.25-335.51	7	5	6	18	16.98	16	88.88	1	5.55	17	94.44	0	0	100
P-5	AAT CGG GCT G	948.68-492.09	4	4	4	12	11.32	10	83.33	1	8.33	11	91.66	0	0	100
P-6	AGT CAG CCA C	941.09-672.41	8	5	5	18	16.98	10	55.55	4	22.22	14	77.77	0	0	100
P-7	TGC CGA GCT G	958.05-330.19	8	8	8	24	22.64	24	100	0	0	24	100	0	0	100
	Over all total		36	35	35	106		90	84.91	8	7.54	98	92.45	0	0	100
	Total bands in each lane %		33.96	33.2	33.2											

Table 9. RAPD-PCR amplification products of DNA extracted from Dry Embryo of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, using seven random primers.

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	948.68-809.92	3	3	3	9	9.00	9	100	0	0	9	100	0	0	100
P-2	TCT GTG CTG G	883.1-813	2	2	2	6	6.00	4	66.66	1	16.66	5	83.33	0	0	100
P-3	GTG ACG TAG G	947.81-745.71	6	7	7	20	20.00	18	90	1	5	19	95	0	0	100
P-4	GGT CCC TGA C	846.81-290.79	4	4	4	12	12.00	8	66.66	2	16.66	10	83.33	0	0	100
P-5	AAT CGG GCT G	947.02-454.4	3	5	3	11	11.00	9	81.82	1	9.1	10	90.91	0	0	100
P-6	AGT CAG CCA C	944.46-809.81	6	6	6	18	18.00	12	66.66	3	16.66	15	83.33	0	0	100
P-7	TGC CGA GCT G	942.77-304.83	8	8	8	24	24.00	24	100	0	0	24	100	0	0	100
	Over all total		32	35	32	100		84	84	8	8	92	92	0	0	100
	Total bands in each lane %		32.00	35.00	32.00											

Table 10. RAPD-PCR amplification products of DNA extracted from Imbibed Embryo of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, using seven random primers.

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	952.14-808.81	4	4	3	11	10.78	9	81.82	1	9.1	10	90.91	0	0	100
P-2	TCT GTG CTG G	879.52-807.62	2	2	2	6	5.88	6	100	0	0	6	100	0	0	100
P-3	GTG ACG TAG G	946.06-758.92	6	6	6	18	17.64	18	100	0	0	18	100	0	0	100
P-4	GGT CCC TGA C	853.71-264.81	4	4	4	12	11.76	8	66.66	2	16.66	10	83.33	0	0	100
P-5	AAT CGG GCT G	965.49-416.26	4	5	3	12	11.76	10	83.33	1	8.33	11	91.66	0	0	100
P-6	AGT CAG CCA C	949.53-808.71	6	6	6	18	17.64	12	61.11	3	16.66	15	83.33	0	0	100
P-7	TGC CGA GCT G	947.84-330.19	8	8	9	25	24.51	23	92	1	4	24	96	0	0	100
	Over all total		34	35	33	102		86	84.31	8	7.84	94	92.2	0	0	100
	Total bands in each lane %															

Table 11. RAPD-PCR amplification products of DNA extracted from radicals of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, using seven random primers .

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	946.96-815.49	3	3	4	10	9.26	8	80	1	10	9	90	0	0	100
P-2	TCT GTG CTG G	883.1-797.12	2	2	2	6	5.56	6	100	0	0	6	100	0	0	100
P-3	GTG ACG TAG G	956.61-764.28	6	6	6	8	16.67	18	100	0	0	18	100	0	0	100
P-4	GGT CCC TGA C	818.63-233.75	3	3	4	10	9.26	8	80	1	10	9	90	0	0	100
P-5	AAT CGG GCT G	950.35-465.39	4	4	5	13	12.04	11	84.62	1	7.69	12	92.31	0	0	100
P-6	AGT CAG CCA C	961.47-809.81	7	7	7	21	19.44	21	100	0	0	21	100	0	0	100
P-7	TGC CGA GCT G	944.46-335.51	10	10	10	30	27.78	22	73.33	4	13.33	26	86.66	0	0	100
	Over all total		35	35	38	108		94	87.04	7	6.48	101	93.52	0	0	100
	Total bands in each lane %		32.41	32.41	35.19											

Table 12. RAPD-PCR amplification products of DNA extracted from pollen grains of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, using seven random primers.

Primers code	Primers sequences (5′ → 3′)	Amplicon lengths (bp)	Total number of scorable bands in each lane			Polymorphic bands								Monomorphic bands		Polymorphism %
						Total bands		Unique		Non -Unique		Polymorphic				
			Lane A	Lane B	Lane C	No	%	No	%	No	%	No	%	No	%	
P-1	CAA ACG TCG G	948.68-814.37	3	4	4	11	10.37	5	45.45	3	27.27	8	72.73	0	0	100
P-2	TCT GTG CTG G	898.78-802.17	2	3	2	7	6.54	5	71.43	1	14.28	6	85.72	0	0	100
P-3	GTG ACG TAG G	960.15-775.1	7	7	7	21	19.81	21	100	0	0	21	100	0	0	100
P-4	GGT CCC TGA C	815.31-256.68	5	5	5	15	14.2	13	86.66	1	6.66	14	93.33	0	0	100
P-5	AAT CGG GCT G	943.7-436.65	5	5	4	14	13.21	12	85.72	1	7.14	13	92.85	0	0	100
P-6	AGT CAG CCA C	961.47-830.91	5	5	5	15	14.2	15	100	0	0	15	100	0	0	100
P-7	TGC CGA GCT G	959.76-387.42	8	8	8	24	22.64	24	100	0	0	24	100	0	0	100
Over all total			35	37	35	107		95	88.78	6	5.61	101	94.39	0	0	100
Total bands in each lane %			32.71	34.57	32.71											

Table 13. Total amplified DNA generated by RAPD-PCR used for identifying the three *P. vulgaris* L. var; A= strike, B=contender, C= wonder.

P. vulgaris varieties	Total bands developmental stages						
	S	DSC	ISC	DEm	IEm	R	PGs
A	36	35	36	32	34	35	35
B	34	35	35	35	35	35	37
C	33	34	35	33	33	38	35

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

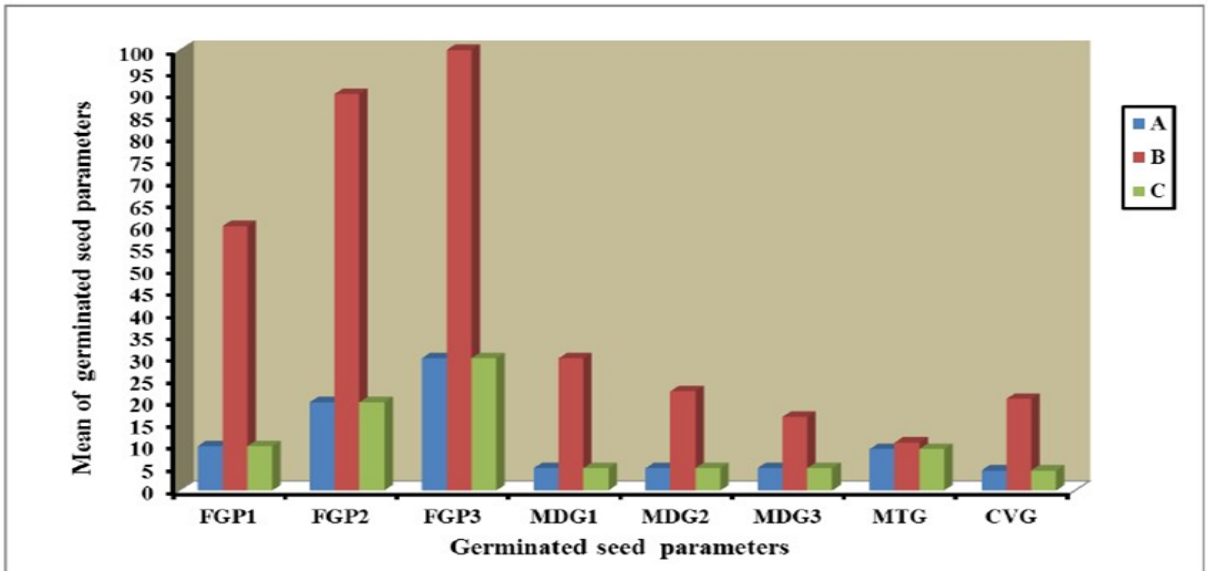

Figure 1. Mean of germination seed parameters of *P. vulgaris* L. var; A= strike, B=contender, C= wonder after 2 days, 4 days, 6 days**.**

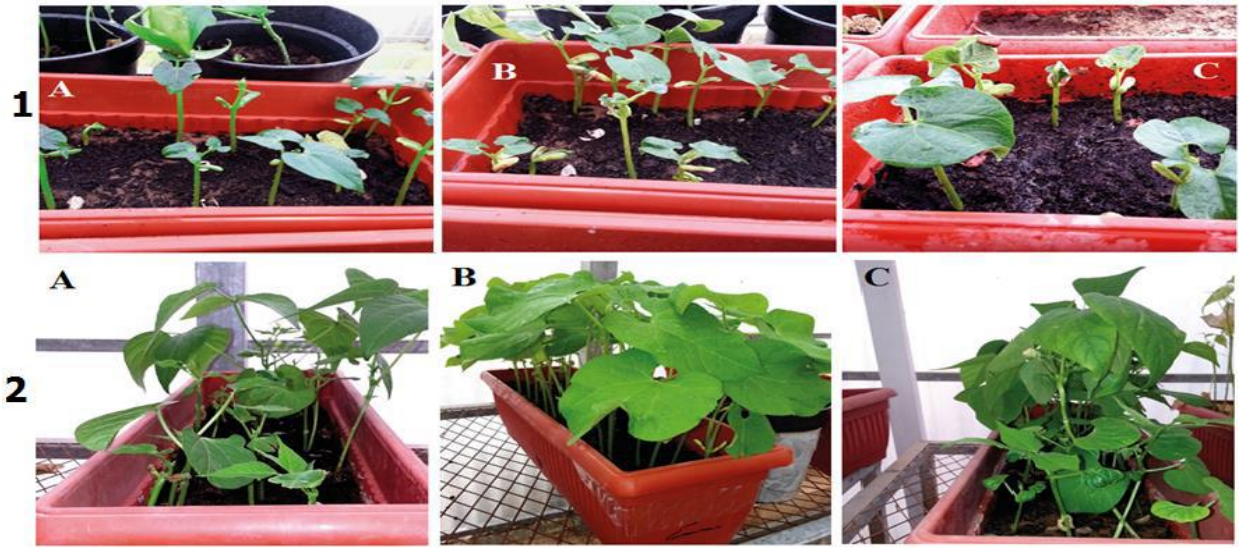

Figure 2. Seedling growth of three *P. vulgaris* L. var; A= strike, B=contender, C= wonder; after 1- (15days) and 2- (30 days) from planting of seeds.

Figure 3. Photos show flower colors and pods containing seeds at flowering and fruiting stages of *P. vulgaris* L. var; A= strike, B=contender, C= wonder.

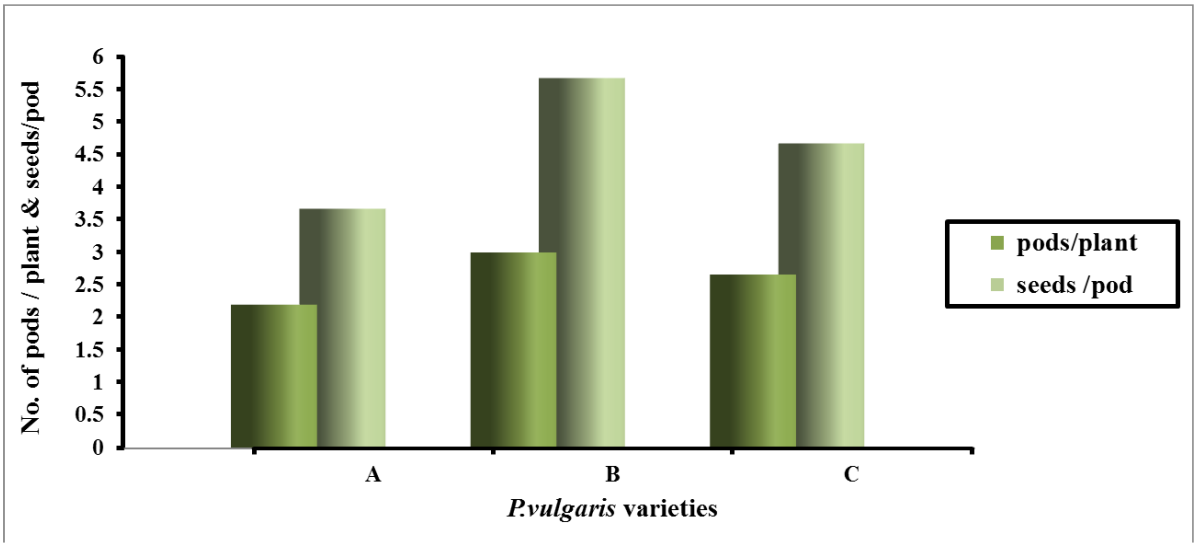

Figure 4 (a). Number. of pods/ plant, seeds / pod of *P. vulgaris* L. var; A- A= strike, B=contender, C= wonder.

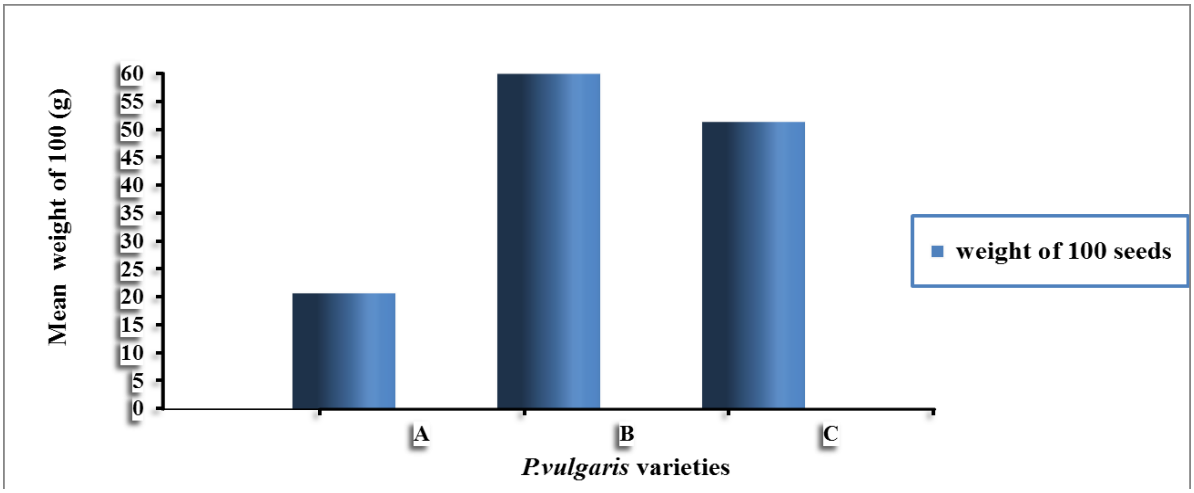

Figure 4 (b). Mean weight of 100 seeds g of *P. vulgaris* L. var; A= strike, B=contender, C= wonder.

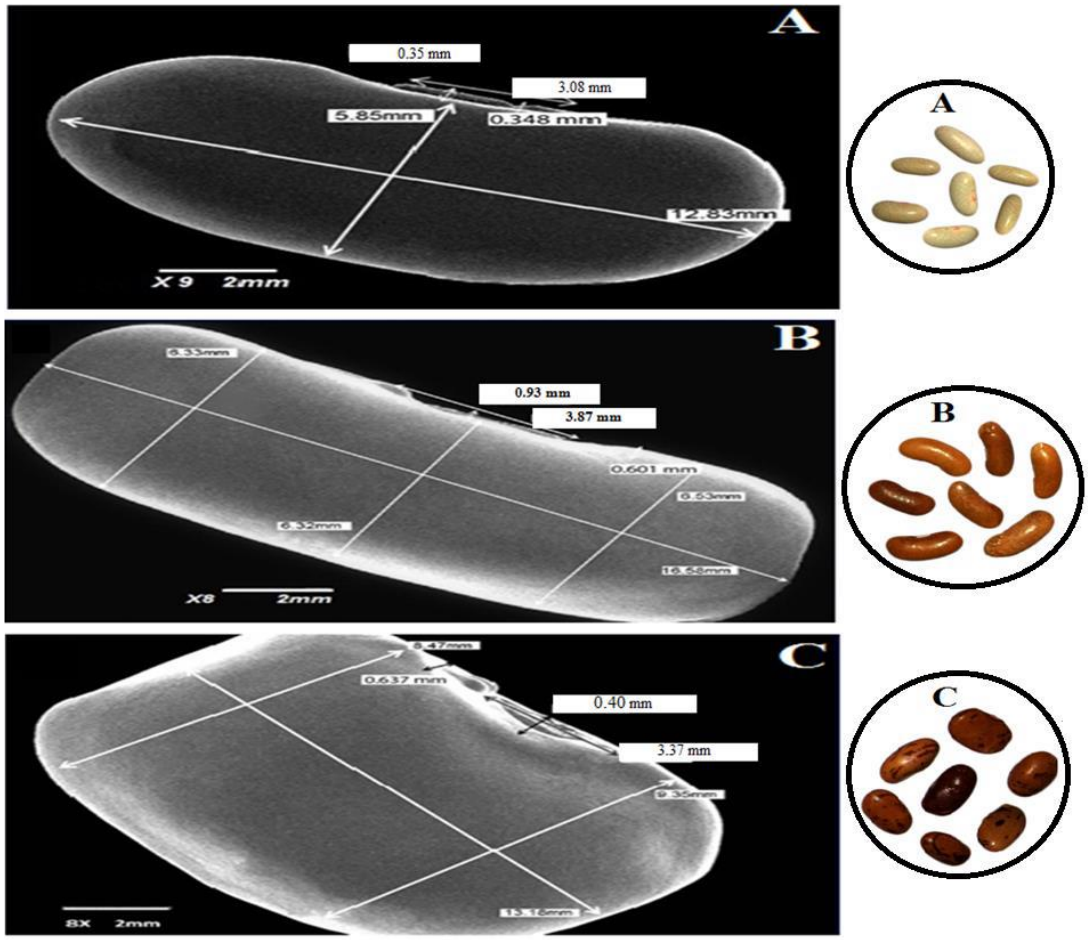

Figure 5. Seed phenotype, seed color, and helium sizes of P. vulgaris L.var; A= strike, B=contender, C= wonder.

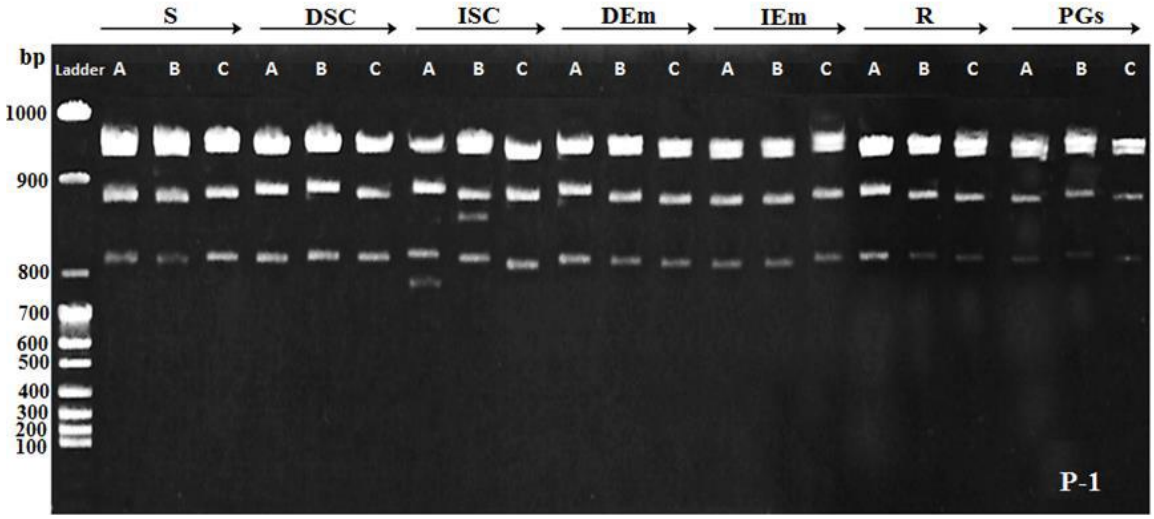

Figure 6. DNA banding pattern of RAPD analysis of DNA of P. vulgaris L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P-1, Code: CAA ACG TCG G.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

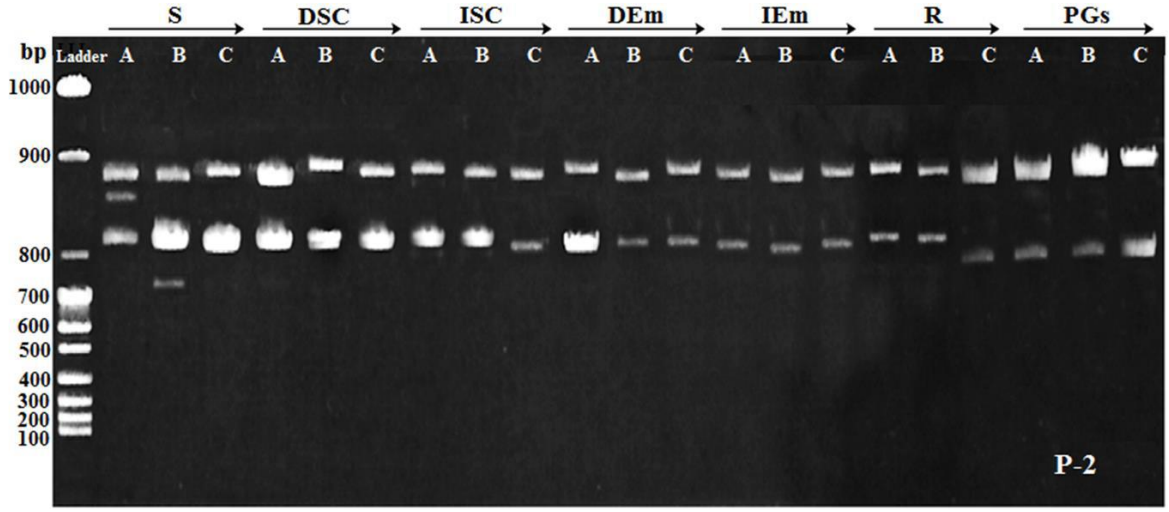

Figure 7. DNA banding pattern of RAPD analysis of DNA of P. vulgaris L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P-2, Code: TCT GTG CTG G.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

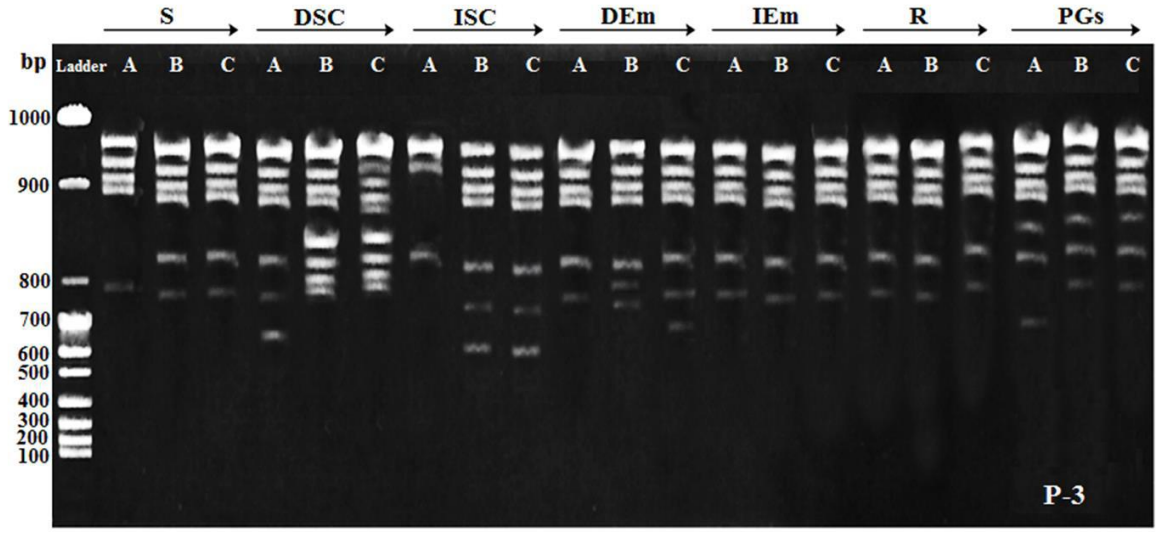

Figure 8. DNA banding pattern of RAPD analysis of DNA of P. vulgaris L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P-3, Code: GTG ACG TAG G.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

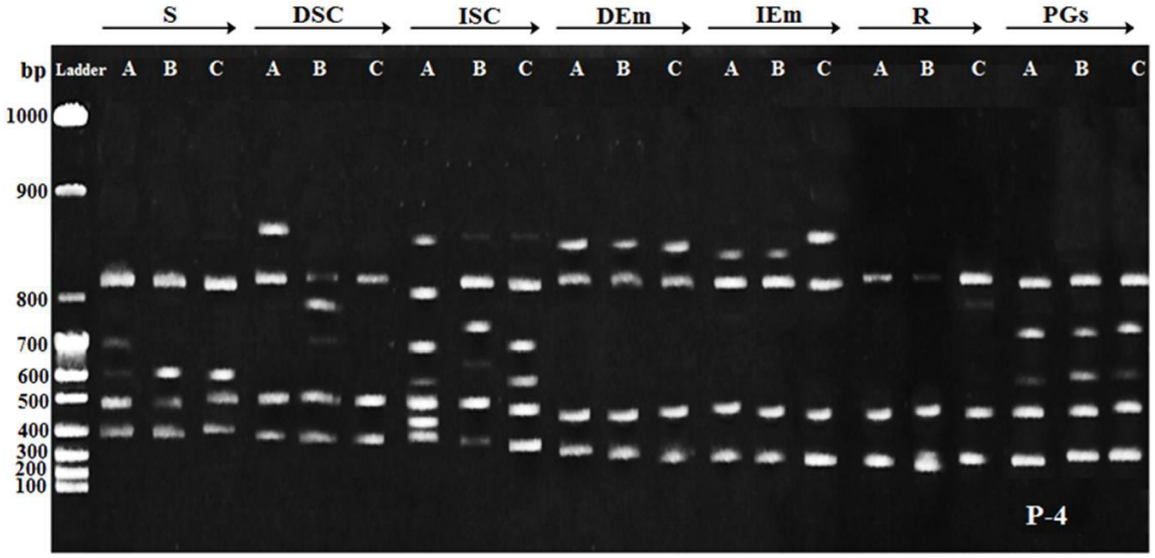

Figure 9. DNA banding pattern of RAPD analysis of DNA of P. vulgaris L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P- 4, Code: GGT CCC TGA C.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

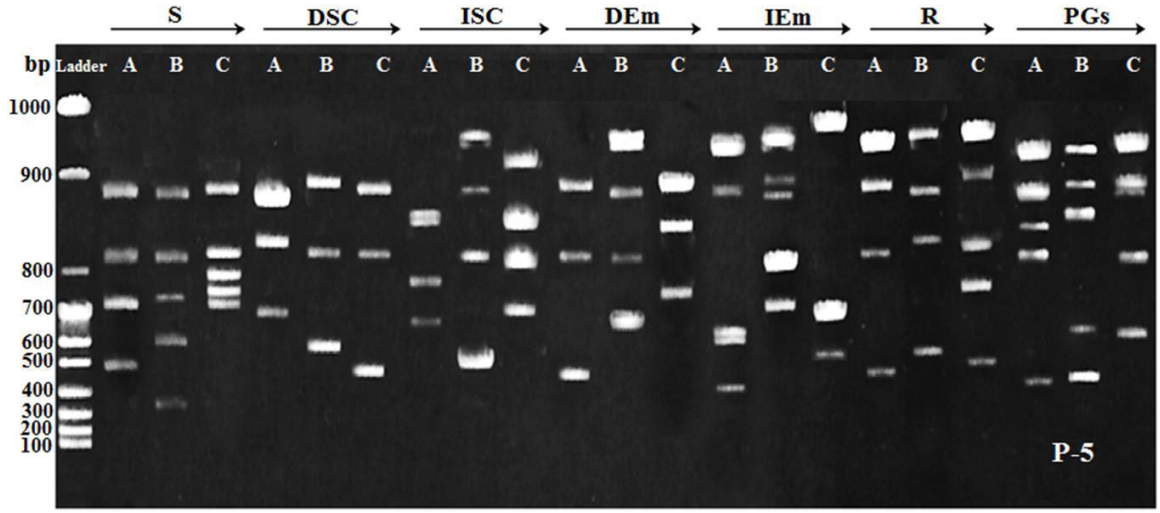

Figure 10. DNA banding pattern of RAPD analysis of DNA of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P-5, Code: AAT CGG GCT G.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

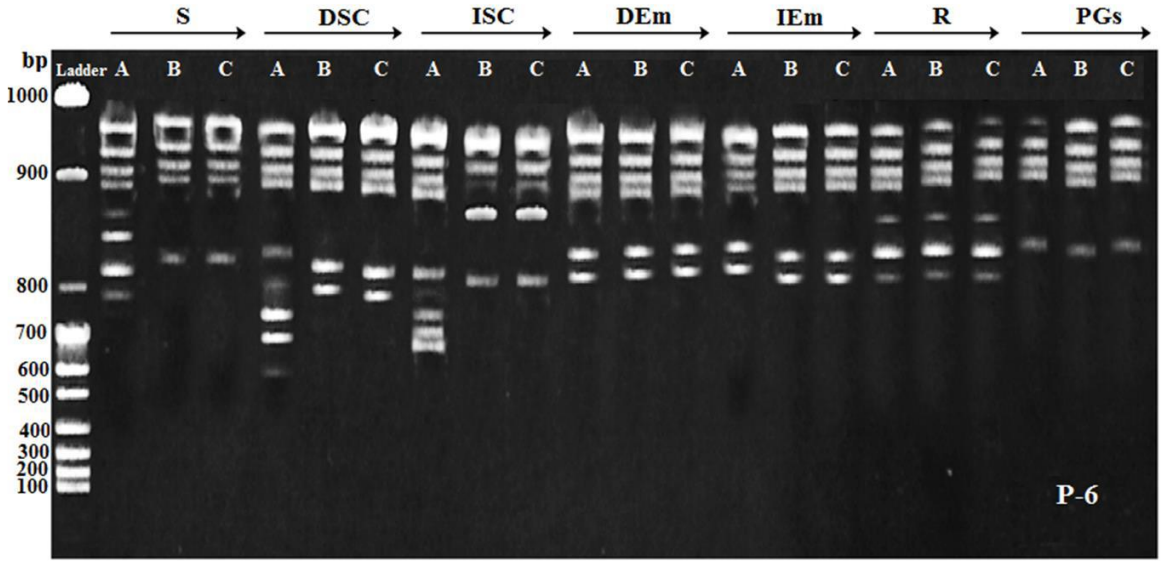

Figure 11. DNA banding pattern of RAPD analysis of DNA of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P-6, Code: AGT CAG CCA C.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

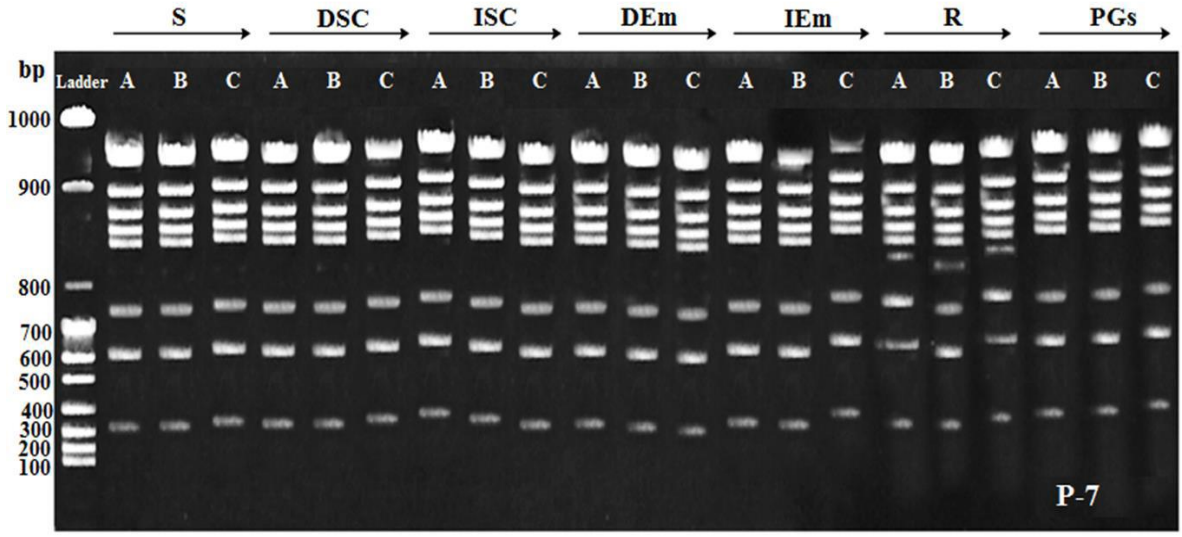

Figure 12. DNA banding pattern of RAPD analysis of DNA of *P. vulgaris* L. var; A= strike, B=contender, C= wonder, during 7 developmental stages using random primer P-7, Code: TGC CGA GCT G.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

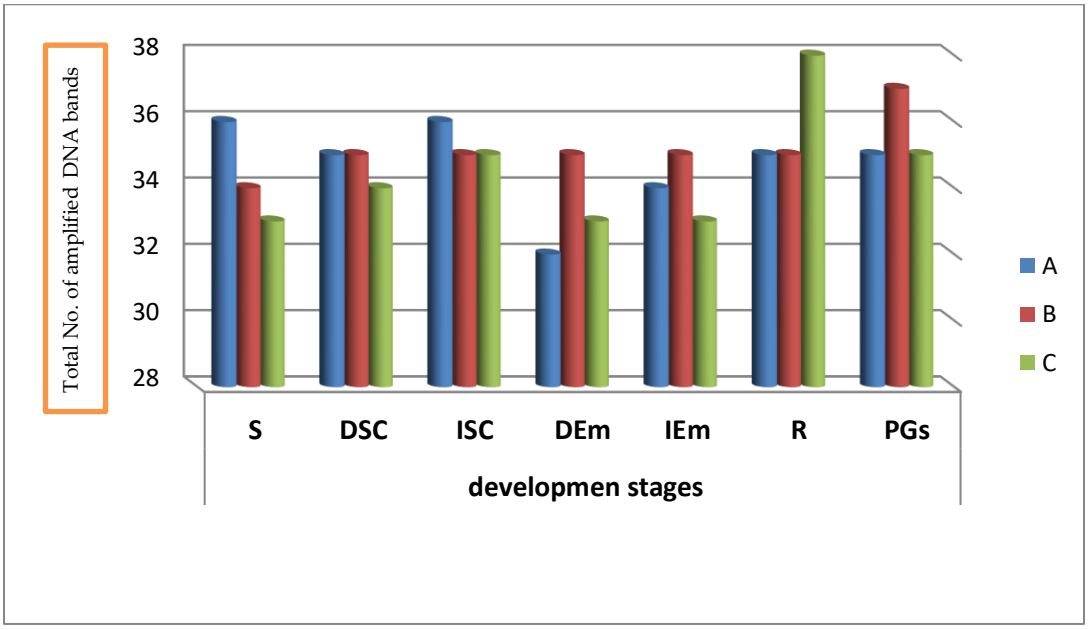

Figure 13. Total amplified DNA products during 7 developmental stages for three *P. vulgaris* L. var; A= strike, B=contender, C= wonder.

Note: S= Full dry Seeds, DSC= Dry Seed Coat, ISC= Imbibed Seed Coat**,** Dem= Dry Embryo, IEm= Imbibed Embryo, R= Radical, PGs= Pollen grains.

التحليل الوراثي والجزيئي لغطاء بذرة وحبوب لقاح نبات الفاصوليا أثناء مراحل تميزهم ونموهم

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الملخص العربي

Phaseolus vulgaris L. (الفاصوليا) من البقوليات ذات القيمة الغذائية العالية وتتميز بتشكيلة واسعة من البذور ذات أنماط و ألوان مختلفة تغطى سمات مظهرية أساسية تميز أصنافها المختلفة كما أن ألوان غطاء البذور محكوم بمجموعة من الجينات داخل أنسجتها تنظم عمليات التخليق الحيوي للفينولات وأصباغ الأنثوسيانينات والتي ترتبط بدورها في إظهار ألوان غطاء البذور. تهدف الدراسة الحالية إلى اختيار 3 أصناف من بذور الفاصوليا وهي كو نتيندر وستريك والوندر المختلفين في لون غطاء البذرة (كريمي، بنى إلى بنى غامق، وأحمر منقط) وتقييم الخصائص المظهرية لهم بإستخدام دليل مظهري على أساس إنبات البذور ونمو البادرات من ناحية ودليل مظهري على أساس غطاء البذور باستخدام الستيريوميكروسكوب والميكروسكوب الإلكتروني من ناحية آخرى. أشارت النتائج إلى أن جميع الواسمات المستخدمة من العلامات الظاهرية إلى الواسمات الجزيئية القائمة على الحمض النووي تختلف في قدرتها على حلها للكشف عن الاختلافات الجينية وتحديد الهوية والهيكل الجيني ونوع ال بيانات التي تولدها لكل نوع. النمط الظاهري وعلامات البروتين كانوا يعتمدون على التعبير الجيني للحمض النووي في منطقة exons (التشفير) فقط. من ناحية آخرى، كانت العلامات المستندة إلى الحمض النووي تعتمد على مناطق التشفير (exons) وغير المشفرة (أنترون) ولا تخضع لعوامل خارجية أعلاه. في الختام، بدا أن العلامات المستندة إلى الحمض النووي هي المقايسة الجزيئية الأنسب لبصمات الأصابع وتقييم البنية الجينية لكل صنف P. vulgaris L. بدقة عالية. أيضًا، P. vulgaris var. مع لون غطاء بذرة بني إلي بني غامق كان أكثر وضوحًا من النوعين الآخرين.

الكلمات المفتاحية: وراثي، التحليل الجزيئي، غطاء البذور، حبوب اللقاح، *Phaseolus vulgaris L.*