# 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 and exhibits a wide variety of seed coat patterns and colors that controlled by a group of specific genes who regulate the flavonol and anthocyanin biosynthetic pathways which in turn responsible for appearing the seed coat colours. This attracted the attention of the current study to select seeds of three varieties of *P. vulgaris* var. strike, contender, and wonder which had different seed coats colours (creamy, Brown to deep brown, and Reddish spotty (pinto) beans respectively). The study 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 current study concluded that all markers used in present study 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. Each technique has its own advantages and limitations. Phenotypical and protein-based markers are dependent on the gene expression of DNA at exons (coding) region only and may be influenced by environmental conditions, tissue specificity, developmental stages and age. On the other hand, the DNA-based markers are dependent on coding (exons) and non-coding (intron) regions and are not governed by above external factors because any changes in them are due to natural mutation within the gene sequence during replication that, in turn, may be repaired. Thus, 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 by which the conservation of the studied plant can be made easy. The current study showed also that *P. vulgaris* var . contender with brown to deep brown seed coat color were more pronounced than the other two varieties in most analyses used in this study.

**Keywords** : Genetic - molecular analysis - seed coats - pollen grains- Phaseolus vulgaris L.

## Introduction

In the angiosperms, fertilization results in the formation of the seed from the ovule. This remarkable transformation involves the activation and coordination of the distinct developmental pathways leading to an embryo, endosperm and seed coat. The seed coat (testa) consists of several layers of specialized maternal cell types that provide an important interface between the embryo and the external environment during embryogenesis, dormancy and germination. Differentiation of the seed coat from the ovule integuments includes some of the most dramatic cellular changes observed during seed development and culminates in the death of the seed coat cells. (Lepiniec *et al.*, 2006)

The main objectives of the present study to acsess the genetic variation among three colored *Phaseolus vulgaris* L. (common bean) seeds using certain markers:

1. To give full identification of plant material and characterization of genetic structure of each *P. vulgaris* variety for knowledge how to conserve them from extinction and to ensure their sustainable use.

2. To analyze proanthocyanidins, anthocyanin amounts in addition to the antioxidant potential based on free radical scavenging.

3. diphenyl-1-picrylhydrazyl, β-carotene-linoleate, total phenolic, and flavonoid contents to correlate between genetic variations among three *Phaseolus vulgaris* varities with the antioxidant potential status.

4. To detect genetic variations among these colored seeds during different seed developmental stages ranged from seed coats to pollen grains.

## REVIEWS OF LITERATURE:

- **Seeds and Seed coat:**

Seeds are the typical propagation units of the flowering plants. They represent the delivery system for the transfer of genetic materials from one generation to the next, trough the sexual reproduction in vascular plant. The seed-producing organisms of the plant kingdom belong to the division Spermatophyta, further classified into 2 sub- divisions: Gymnospermae (gymnosperms) and Angiospermae (angiosperms). The angiosperms are divided into 2 classes:

Monocotyledoneae and Dicotyledoneae (Linkies *et al.*, 2010).

It is a goal of agricultural research to accelerate the development of seed quality and yield and to diversify their traits to satisfy more of our needs. Technologies such as genomics, proteomics, and metabolomics promise to accelerate our understanding of seeds and thus open new possibilities for uses. Emerging technologies for generating transgenic plants provide an opportunity for enhancing existing seed traits and for adding value to seeds (Moise *et al.*, 2005).

- **Legumes and *Phaseolus vulgaris* L. (common bean):**

Seed development in legume is highly related to nutrient metabolism and its transport and the phases of seed development are well established in many legume species ,Seed development proceeds through three distinct phases: histodifferentiation (embryogenesis), seed filling, and desiccation (Weber *et al.*, 2005).

Recently, common bean is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals with potential health benefits such as fiber, polyphenolic compounds, lectins, unsaturated fatty acids, trypsin inhibitors, phytic acid, among others (Guzmán-Maldonado *et al.*, 1998). Important biological activities have been described for fiber, polyphenolic compounds (Queiroz-Monici *et al*., 2005); and phytic acid from common beans like enhancement of the bifidogenic effecteffects; as well as an antiproliferative effect on transformed cells (Aparicio-Fernández *et al*., 2006).

- **Polyphenolic compounds, anthocyanins and Antioxidants:**

The seed color of beans is determined by the presence of polyphenolic compounds. Polyphenolic compounds are famous because they are known to reduce diseases caused by reactive oxygen and nitrogen species (Maestri *et al.*, 2006; Miliauskas, 2006).

Dzomba *et al.* (2013) investigated anthocyanin, proanthocyanidin and antioxidant activity of five (black, brown, white, brown and black spotted) *Phaseolus vulgaris* L. (common bean) species grown in Mashonaland Central, Zimbabwe. They concluded that the five common bean species exhibited variations in anthocyanins, proanthocyanidin and antioxidant activity.

- **Pollen grains:**

In flowering plants, Pollen grains represent the highly reduced haploid male gametophyte generation in flowering plants, consisting of just two or three cells when released from the anthers. Their role is to deliver twin sperm cells to the embryo sac to undergo fusion with the egg and central cell. This double fertilization event along with the functional specialization of the male gametophyte, are considered to be key innovations in the evolutionary success of flowering plants (Borg *et al*.,2009)

Borg *et al*. (2009) also reported that the male gametophyte (or pollen grain) plays a vital role in plant fertility, and crop production through the generation and delivery of the male gametes to the embryo sac for double fertilization. Pollen grains also play an important role in formation of phenotypic characters of the seed after fertilization. The haploid male gametophyte generation of flowering plants consists of two- or three-celled pollen grains. This functional specialization is thought to be a key factor in the evolutionary success of flowering plants. Moreover, pollen ontogeny is also an attractive model in which to dissect cellular networks that control cell growth, asymmetric cell division and cellular differentiation.

- **Biomarkers used in Genetic variation:**

Seed storage proteins are deposited in relatively large quantities in mature seeds and typically remain more stable than other plant tissues until they germinate (Mirali *et al.*, 2007).

Additionally, it is a method commonly used to investigate genetic diversity and to classify plant varieties (Kakaei and Kahrizi, 2011), as genetic markers for genetic variation, to detect genetic diversity in cultivated and wild plant species, and to provide information on phylogenetic relationships among accessions (Kumar and Tata, 2010; Emre, 2011).

DNA markers are independent markers that segregate as single genes; their environmental stability make them ideal tools for studying plants (Kumar *et al*., 2009). They are used in several techniques, including RAPD-PCR, which is a fast technique for revealing genetic variation and reflecting underlying genetic variation (Gonçalves *et al*., 2008).

## MATERIALS AND METHODS:

- **MATERIALS:**

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

**2.** 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.

- **METHODS:**

**(1) Phenotypic parameters:**

**A. 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 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 for determination of 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 taken into consideration were the final germination percent (FGP) as per (ISTA, 1985); means daily germination (MDG) is an index of daily germination rate (Scott *et al.,* 1984); Means time of germination is an index for germination rate (MTG) as per (Ellis and Roberts, 1981); and coefficient of velocity of germination (CVG) is an index for germination speed (Maguire, 1962).

**B.** 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 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.

**C. Determination of Seedling growth parameters of *P. Vulgaris*:**At seedling stage and 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 Vashisth and Nagarajan (2010)

**(2) Statistical analyses:**

Each experiment for seed germination parameters and seedling growth parameters was carried out in t riplicates. Data are expressed as means ± standard deviation (SD). The obtained data were statistically analyzed by using one way ANOVA, probability level was 0.05.

**RESULTS:**

**Results of seedling growth parameters:**

The results presented in Table (1) 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.

Table (1): Seedling growth parameters of *P.vulgaris* L.var; A- strike, B- contender, C- wonder values are means ±SD

| Code | | | | | | |
|---|---|---|---|---|---|---|
| | | | | Mean of Seedling growth parameters ±SD after | | |
| | | | | 15 days | | |
| | Mean Lengths (cm) | Root | A | B | C | LSD at 5% |
| | | | 12.00±1.32 | 25.36*±2.56 | 14.16±1.25 | 3.62 |
| | | Shoot | 10.83±0.72 | 15.43*±1.10 | 10.66±1.75 | 2.53 |
| | | Seedling | 22.83±2.03 | 40.76*±2.00 | 23.5±3.12 | 4.86 |
| | Mean Root /Shoot Ratios | | 1.10±0.55 | 1.64*±0.26 | 1.21±0.25 | 0.423 |
| | Leaf parameters | No. of leaves/plant | 2 | 2 | 2 | 0.00 |
| | | Surface area ($cm^2$) | 9.26±1.41 | 64.32*±2.42 | 20.63±1.64 | 3.71 |
| | Seedling Dry Weight (g) | | 1.24±0.31 | 5.46*±0.38 | 2.22±0.19 | 0.616 |
| | (VI) I of Seedling length | | 599.85 | 4076.00 | 705.00 | —— |
| | (VI) II of Seedling Dry Weight | | 33.05 | 546 | 66.60 | —— |
| | Mean Lengths(cm) | Root | 12.40±1.73 | 27.00*±2.50 | 12.70±1.49 | 3.89 |
| | | Shoot | 8.83±0.76 | 18.56*±1.60 | 8.66±0.28 | 2.05 |
| | | Seedling | 21.23±0.97 | 45.23*±1.56 | 20.16±3.18 | 4.23 |

| | | | | | |
|---|---|---|---|---|---|
| 0.61 | 1.35±0.36 | 1.49*±0.24 | 1.41±0.32 | Mean Root /Shoot ratios | |
| 0.00 | 3 | 3 | 3 | No. of leaves/plant | Leaf parameters |
| 3.81 | 24.26±1.11 | 63.16*±1.78 | 23.71±2.57 | Surface area ($cm^2$) | |
| 1.46 | 2.71±0.59 | 4.93*±1.05 | 2.1±0.39 | Seedling Dry Weight (g) | |
| — | 603 | 4523 | 565.99 | (VI) I of Seedling length | |
| — | 81.30 | 493 | 58.38 | (VI) II of seedling Dry Weight | |

**Phenotypic features of flower color and fruiting criteria:**

The results presented in Table (2) 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.

**Table (2):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. |

** Seeds Sizes according with Singh *et al.* (1991a) weight of 100 seeds in grams (Small – 100 seeds <25 g; Medium– 100 seeds ≥ 25 g to ≤ 40 g; Large – 100 seeds

> 40 g).

**Results of Macro and microphenotypical aspects of *P. Vulgaris* L.varieties*:***

Macro-phenotypical aspects of three *P.vulgaris* L. varieties using Stereomicroscopy model are given in Table (3). Data obtained by stereomicroscope investigation showed 8 criteria based on the seed characters including, shape,Texture, Phenotypic seed color, Average seed sizes by Length x width (L×W) mm, Position, and Hilum sizes by Length x width (L×W) mm.

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

| Code | Seed Macro-phenotypical aspects | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Shape | Texture | Phenotypic seed color | Seed size ±SD | | | Hilum | |
| | | | | 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** | Cylinderal 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 |

**Micro-phenotypical aspects using Scanning Electron Microscope (SEM):**

Micro-phenotypical aspects of three *P. vulgaris* L.varieties are given in Table (4) .Data obtained by using SEM investigation showed 8 criteria based on Overall seed coat pattern, Epidermal cell, anticinal walls and periclinal walls.

**Table.(4): Micro-phenotypical aspects *P. vulgaris* L.var.; A- strike, B-contender, C- wonder using Scanning Electron Microscope (SEM) values are means ± SD (n=3).**

| Code | Seed Coat Phenotypical aspects | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Overall seed coat pattern | Epidermal cell | Anticlinal walls | | | Periclinal walls | | |
| | | | Shape | Thickness | Texture + Height | Surface | Texture | Average cell size Length x width (L×W) µm±SD |
| **A** | Irregular Reticulatecrimpy | regular rounded-heteromorphic | Slightly raised andirregular elevations | Slightly thick and irregular | Rough and raised | Slightly concave | Rough with Wrinkled surface | 61.40±0.56 |
| **B** | RegularReticulate | Irregular rounded to polygonal heteromorphic | Straight irregular | thick and irregular | Rough and straight | Flat with Lightly protrusion | Smooth with fin protruded | 47.79±0.41 |
| **C** | RegularReticulate | Irregular triangle to polygonal heteromorphic | Straight regular | Very thick and irregular | Smooth and slightly raised | Flat to slightly concave | Rough granulated | 52.04±0.52 |

**Anthocyanin, proanthocyanidins, and antioxidant potential assays of three *P. vulgaris* L.varieties:**

**Table (5) Antioxidant capacity, total phenolic, flavonoid contents, contents of anthocyanin and proanthocyanidin pigments seeds in *P. vulgaris* L.var; A-strike B-contender C- wonder values are means ±SD (n=3).**

| Code | Antioxidant activity parameters | | | | Anthocyanin and proanthocyanidin pigments | |
|---|---|---|---|---|---|---|
| | DPPH Scavenging % | β-Carotene antioxidant Activity% | Phenol content (GAE µg/mg) | Flavonoid content (QE µg/mg) | Anthocyanin Contents (mg/100g) | Proanthocyanidin contents (mg / 100g catechin) |
| **A** | 35.47± 0.47 | 51.32± 2.40 | 29.45± 0.02 | 79.55± 1.12 | 2.05±0.01 | 0. 20±0.00 |
| **B** | 40.16± 0.04 | 53.69± 2.30 | 33.08± 0.04 | 82.41± 2.01 | 9.55±0.05 | 1.8 ±0.01 |
| **C** | 36.08± 0.21 | 49.36± 2.00 | 31.79± 0.21 | 78.42± 1.60 | 6.19±0.02 | 1.10±0.01 |

The results of anthocyanin and proanthocyanidin determination are shown in Table (5) . Brown to deep brown *P. vulgaris* L.of contender variety exhibited the greatest anthocyanins and proantho cyanidins content, 9.55±0.05mg/100g and 1.80 ±0.01mg/100g, respectively, while creamy of strike varietyshowed the least anthocyanins and proanthocyanidins content 2.05±0.01mg/100g and 0.20±0.00mg/100g, respectively. Anthocyanins and proanthocyanidins content decreased significantly in the order: Brown to deep brown>Reddish spotty (pinto)> creamy.

**Biomarkers analyses of seeds of three *P. vulgaris* L.varieties during developmental stages:**

The electrophoretic banding patterns of total seed protein as revealed by SDS-PAGE were used to detect the genetic variations among dry seeds of three *P. vulgaris* L.varieties during developmental stages remembered above as shown in Table (6). SDS-PAGE analysis revealed 56 polypeptide bands with different molecular weights ranging from 13.46 to 206.44KDa. Out of which, 51 bands were polymorphic with value 91.10% (46 bands were unique, 5 bands were non- unique with values 82.14% and 8.92% respectively) while monomorphic bands absent. SDS-PAGE analysis generated highly polymorphism values of 100%.The maximum number of bands (22 bands) was found at strike variety with value of 39.28%. The minimum number of bands (16 bands) found at contender variety with value of 28.57% as shown in Table (6).

| Table (6): Electrophoretic banding analysis of seed storage proteins of *P. vulgaris* L.var.; A- strike, B- contender, and C-wonder by documentation system Model (Gel Doc Bio Rad system 2000) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lane Rows | Molecular weights (KDa) | Developmental Stage of three varieties of *P. vulgaris* L. | | | | | | |
| | | Full dry Seeds | | | | | | |
| | | Code of lanes | | | | | | Type of bands |
| | | Lane A | | Lane B | | Lane C | | |
| | | KDa | % | KDa | % | KDa | % | |
| 1 | 206.44 | 0 | - | 0 | - | 1 | 2.94 | U |
| 2 | 174.24 | 1 | 4.54 | 1 | 4.31 | 0 | - | Non-U |
| 3 | 129.14 | 0 | - | 0 | - | 1 | 0.631 | U |
| 4 | 115.53 | 0 | - | 1 | 0.386 | 1 | 0.82 | Non-U |
| 5 | 114.08 | 1 | 10.6 | 0 | - | 0 | - | U |
| 6 | 112.63 | 0 | - | 0 | - | 1 | 0.631 | U |
| 7 | 109.73 | 0 | - | 0 | - | 1 | 2.96 | U |
| 8 | 109 | 0 | - | 1 | 2.92 | 0 | - | U |
| 9 | 107.55 | 1 | 5.89 | 0 | - | 1 | 0.82 | Non-U |
| 10 | 105.38 | 1 | 1.85 | 0 | - | 0 | - | U |
| 11 | 103.93 | 0 | - | 0 | - | 1 | 0.631 | U |
| 12 | 101.75 | 1 | 1.78 | 0 | - | 0 | - | U |
| 13 | 101.03 | 0 | - | 1 | 3.44 | 0 | - | U |
| 14 | 98.13 | 0 | - | 0 | - | 1 | 10.2 | U |
| 15 | 97.24 | 0 | - | 1 | 8.66 | 0 | - | U |
| 16 | 96.47 | 0 | - | 0 | - | 1 | 12.3 | U |
| 17 | 95.69 | 0 | - | 1 | 28.1 | 0 | - | U |
| 18 | 95.53 | 0 | - | 0 | - | 1 | 10.9 | U |
| 19 | 94.91 | 0 | - | 0 | - | 1 | 17.9 | U |
| 20 | 92.72 | 1 | 8.14 | 0 | - | 0 | - | U |
| 21 | 77.12 | 1 | 3.8 | 0 | - | 0 | - | U |

**Table (6): Electrophoretic banding analysis of seed storage proteins of *P. vulgaris* L.var.; A- strike, B- contender, and C- wonder by documentation system Model (Gel Doc Bio Rad system 2000)**

| Lane Rows | Molecular weights (KDa) | Developmental Stage of three varieties of *P. vulgaris* L. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Full dry Seeds | | | | | | |
| | | Code of lanes | | | | | | Type of bands |
| | | Lane A | | Lane B | | Lane C | | |
| | | KDa | % | Kda | % | KDa | % | |
| 22 | 66.2 | 0 | - | 1 | 12.5 | 0 | - | U |
| 23 | 64.19 | 0 | - | 0 | - | 1 | 10.2 | U |
| 24 | 63.18 | 1 | 10.4 | 0 | - | 0 | - | U |
| 25 | 61.16 | 0 | - | 1 | 9.2 | 0 | - | U |
| 26 | 59.14 | 0 | - | 1 | 8.56 | 0 | - | U |
| 27 | 57.12 | 1 | 8.26 | 0 | - | 0 | - | U |
| 28 | 56.11 | 0 | - | 0 | - | 1 | 6.23 | U |
| 29 | 53.08 | 0 | - | 1 | 3.78 | 0 | - | U |
| 30 | 52.07 | 1 | 1.73 | 0 | - | 0 | - | U |
| 31 | 51.06 | 0 | - | 0 | - | 1 | 5.03 | U |
| 32 | 48.03 | 0 | - | 1 | 2.29 | 0 | - | U |
| 33 | 47.02 | 0 | - | 0 | - | 1 | 3.27 | U |
| 34 | 46.01 | 1 | 10.4 | 0 | - | 0 | - | U |
| 35 | 45 | 0 | - | 1 | 3.44 | 1 | 3.84 | NonU |
| 36 | 43.6 | 1 | 8.96 | 0 | - | 0 | - | U |
| 37 | 38 | 0 | - | 0 | - | 1 | 5.06 | U |
| 38 | 33.8 | 0 | - | 0 | - | 1 | 1.08 | U |
| 39 | 31 | 0 | - | 1 | 2.92 | 0 | - | U |

**Table (6): Electrophoretic banding analysis of seed storage proteins of *P. vulgaris* L.var.; A- strike, B- contender, and C- wonder by documentation system Model (Gel Doc Bio Rad system 2000)**

| Lane Rows | Molecular weights )KDa( | Developmental Stage of three varieties of *P. vulgaris* L. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Full dry Seeds | | | | | | |
| | | Code of lanes | | | | | | Type of bands |
| | | Lane A | | | | | | |
| | | KDa | % | KDa | % | KDa | % | |
| 40 | 30.48 | 1 | 6.28 | 0 | - | 0 | - | U |
| 41 | 29.95 | 0 | - | 0 | - | 1 | 1.00 | U |
| 42 | 28.37 | 1 | 4.37 | 0 | - | 0 | - | U |
| 43 | 26.78 | 0 | - | 1 | 3.44 | 0 | - | U |
| 44 | 26.25 | 0 | - | 0 | - | 1 | 2.94 | U |
| 45 | 25.73 | 1 | 0.196 | 0 | - | 0 | - | U |
| 46 | 19.61 | 1 | 0.839 | 0 | - | 0 | - | U |
| 47 | 19.14 | 0 | - | 1 | 6.16 | 1 | 7.5 | Non-U |
| 48 | 17.72 | 1 | 0.944 | 0 | - | 0 | - | U |
| 49 | 16.3 | 0 | - | 0 | - | 1 | 6.8 | U |
| 50 | 15.82 | 0 | - | 1 | 6.25 | 0 | - | U |
| 51 | 13.46 | 1 | 0.968 | 0 | - | 0 | - | U |
| Total Bands in each lane | | 18 | | 16 | | 22 | | Polymorphism% |
| Total Bands in all lane | | 56 | | | | | | |
| % | | 32.93 | | 28.57 | | 39.28 | | |
| Types and frequency of bands and Polymorphism | | Unique bands | | Non-Unique bands | | Polymorphicbands | Monomorphic bands | |
| | | No. | % | No. | % | No. | | % | No. | % | 100 |
| | | 46 | 82.14 | 5 | 8.92 | 51 | | 91.1 | 0 | 0 | |

- **Analysis of amino acid composition of seeds of *P. vulgaris* L. varieties by High-Performance Liquid Chromatography (HPLC-Analysis):**

The results of EAA and non-essential (NEAAs) amino acid compositions of the raw seeds of three *P. vulgaris* L. varieties used in current study shown in Table (7) . HPLC analysis revealed the presence of 17 amino acids, 9 of which are essential in humans.

**Table (7): Amounts of essential and Non-essential amino acids of developmental stages of *P. vulgaris* L.var; A-strike, B-contender , and C-wonder .**

| | Seed developmental stages | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Amino acids (gm/100gm protein)** | **Seed** | | | **Dry seed coat** | | | **Imbibed seed coat** | | | **Dry embryo** | | | **Imbibed embryo** | | | **Radicals** | | | **Pollen grains** | | |
| | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** |
| **Essential Amino Acids** | | | | | | | | | | | | | | | | | | | | | |
| **Arginine** | 16.81 | 14.61 | 13.55 | 10.67 | 9.66 | 13.25 | 8.67 | 7.02 | 4.07 | 18.85 | 19.05 | 18.19 | 17.37 | 16.19 | 17.00 | 17.85 | 18.74 | 19.68 | 12.99 | 11.83 | 10.78 |
| **Histidine** | 4.55 | 5.17 | 4.09 | 4.16 | 2.92 | 4.00 | 2.62 | 2.12 | 1.91 | 5.51 | 5.61 | 5.36 | 5.12 | 4.77 | 5.01 | 5.76 | 6.05 | 6.35 | 4.82 | 4.40 | 4.00 |
| **Isoleucine** | 3.14 | 3.22 | 3.05 | 2.89 | 2.17 | 2.76 | 1.95 | 1.58 | 1.32 | 3.80 | 3.66 | 3.50 | 3.34 | 3.11 | 3.27 | 2.87 | 3.02 | 3.17 | 2.41 | 2.19 | 2.00 |
| **Leucine** | 1.23 | 1.55 | 1.11 | 1.13 | 0.79 | 1.08 | 0.71 | 0.58 | 0.52 | 1.49 | 1.56 | 1.49 | 1.42 | 1.33 | 1.39 | 1.46 | 1.53 | 1.61 | 1.22 | 1.12 | 1.02 |
| **Lysine** | 4.15 | 4.61 | 4.02 | 3.64 | 2.87 | 3.65 | 2.57 | 2.08 | 1.74 | 5.03 | 5.26 | 5.02 | 4.80 | 4.47 | 4.69 | 4.93 | 5.18 | 5.43 | 4.13 | 3.76 | 3.43 |
| **Methionine** | 4.16 | 4.25 | 4.04 | 3.83 | 2.88 | 3.66 | 2.59 | 2.09 | 1.75 | 5.04 | 5.46 | 5.21 | 4.98 | 4.64 | 4.87 | 5.12 | 5.37 | 5.64 | 4.29 | 3.91 | 3.56 |
| **Phenylalanine** | 2.33 | 2.61 | 2.31 | 2.14 | 1.65 | 2.05 | 1.48 | 1.20 | 0.98 | 2.82 | 3.05 | 2.91 | 2.78 | 2.59 | 2.72 | 2.86 | 3.00 | 3.15 | 2.39 | 2.18 | 1.99 |
| **Threonine** | 5.79 | 5.33 | 5.11 | 5.33 | 3.64 | 5.10 | 3.27 | 2.65 | 2.43 | 7.01 | 8.22 | 7.85 | 7.50 | 6.99 | 7.34 | 5.65 | 5.93 | 6.23 | 4.73 | 4.31 | 3.93 |
| **Valine** | 2.57 | 2.73 | 2.51 | 2.36 | 1.79 | 2.26 | 1.61 | 1.30 | 1.08 | 3.11 | 3.33 | 3.18 | 3.04 | 2.83 | 2.97 | 3.12 | 3.28 | 3.44 | 2.61 | 2.38 | 2.17 |
| **Total** | 44.73 | 44.08 | 39.79 | 36.15 | 28.37 | 37.81 | 25.47 | 20.62 | 15.8 | 52.66 | 55.2 | 52.71 | 50.35 | 46.92 | 49.26 | 49.62 | 52.1 | 54.7 | 39.59 | 36.08 | 32.88 |
| **Sum** | 128.6 | | | 102.33 | | | 61.89 | | | 160.57 | | | 146.53 | | | 156.42 | | | 108.55 | | |
| **%** | 34.78 | 34.27 | 30.94 | 35.32 | 27.72 | 36.94 | 41.15 | 33.31 | 25.52 | 32.79 | 34.37 | 32.82 | 34.36 | 32.02 | 33.61 | 31.72 | 33.30 | 34.96 | 36.47 | 33.23 | 30.29 |
| **Non- Essential Amino Acids** | | | | | | | | | | | | | | | | | | | | | |
| **Alanine** | 3.92 | 3.59 | 3.42 | 3.61 | 2.44 | 3.45 | 2.19 | 1.77 | 1.65 | 4.75 | 5.41 | 5.17 | 4.93 | 4.60 | 4.83 | 5.07 | 5.32 | 5.59 | 4.25 | 3.87 | 3.53 |
| **Aspart** | 3.08 | 3.22 | 2.88 | 3.51 | 2.05 | 2.71 | 1.84 | 1.49 | 1.29 | 3.73 | 3.99 | 3.81 | 3.64 | 3.39 | 3.56 | 3.74 | 3.93 | 4.12 | 3.13 | 2.85 | 2.60 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ic acid | | | | | | | | | | | | | | | | | | | | |
| Glutamic acid | 6.17 | 5.59 | 5.41 | 6.51 | 3.86 | 5.43 | 3.46 | 2.80 | 2.59 | 7.47 | 8.05 | 7.69 | 7.34 | 6.84 | 7.18 | 5.68 | 4.50 | 3.56 | 2.81 | 2.56 | 2.33 |
| Cysteine | 3.79 | 3.57 | 3.51 | 3.49 | 2.50 | 3.34 | 2.25 | 1.82 | 1.59 | 4.59 | 5.64 | 5.39 | 5.14 | 4.79 | 5.03 | 5.29 | 5.55 | 5.83 | 4.43 | 4.03 | 3.68 |
| Proline | 1.15 | 1.82 | 1.19 | 1.06 | 0.85 | 1.01 | 0.76 | 0.62 | 0.48 | 1.39 | 1.44 | 1.38 | 1.31 | 1.22 | 1.29 | 1.35 | 1.42 | 1.49 | 1.13 | 1.03 | 0.94 |
| Tyrosine | 2.08 | 2.33 | 1.95 | 1.91 | 1.39 | 1.83 | 1.25 | 1.01 | 0.87 | 2.52 | 2.65 | 2.53 | 2.42 | 2.25 | 2.36 | 2.48 | 2.61 | 2.74 | 2.08 | 1.90 | 1.73 |
| Asparagine | 3.33 | 3.65 | 3.39 | 4.13 | 2.42 | 2.93 | 2.17 | 1.76 | 1.40 | 4.03 | 4.61 | 4.40 | 4.20 | 3.92 | 4.11 | 4.32 | 4.54 | 4.76 | 3.62 | 3.30 | 3.00 |
| Glutamine | 3.91 | 3.55 | 3.49 | 4.55 | 2.49 | 3.44 | 3.02 | 2.44 | 1.64 | 4.74 | 4.69 | 4.48 | 4.28 | 3.99 | 4.19 | 3.68 | 3.87 | 4.06 | 3.09 | 2.81 | 2.56 |
| Total | 27.43 | 27.32 | 25.24 | 28.77 | 18 | 24.14 | 16.94 | 13.71 | 11.51 | 33.22 | 36.48 | 34.85 | 33.26 | 31 | 32.55 | 31.61 | 31.74 | 32.15 | 24.54 | 22.35 | 20.37 |
| Sum | 79.99 | | | 70.91 | | | 42.16 | | | 104.55 | | | 96.81 | | | 95.50 | | | 67.26 | | |
| % | 34.29 | 34.15 | 31.55 | 40.57 | 25.38 | 34.04 | 40.18 | 32.51 | 27.30 | 31.77 | 34.89 | 33.33 | 34.35 | 32.02 | 33.62 | 33.09 | 33.23 | 33.66 | 36.48 | 33.22 | 30.28 |
| Total of EAAs and non-EAA | 72.16 | 71.4 | 65.03 | 64.92 | 46.37 | 61.95 | 42.41 | 34.33 | 27.31 | 85.88 | 91.68 | 87.56 | 83.61 | 77.92 | 81.81 | 81.23 | 83.84 | 86.85 | 64.13 | 58.43 | 53.25 |
| Sum | 208.59 | | | 173.24 | | | 104.05 | | | 265.12 | | | 243.34 | | | 251.92 | | | 175.81 | | |
| % | 34.59 | 34.22 | 31.17 | 37.05 | 26.76 | 35.75 | 40.75 | 32.99 | 26.24 | 32.39 | 34.58 | 33.02 | 34.35 | 32.02 | 33.61 | 32.24 | 33.28 | 34.47 | 36.47 | 33.23 | 30.28 |

*Phaseolus vulgaris* L. (Common bean) is a valuable and highly nutritious food legume and exhibits a wide variety of seed coat patterns and colors that controlled by a group of specific genes who regulate the flavonol and anthocyanin biosynthetic pathways which in turn responsible for appearing the seed coat colours. This attracted the attention of the current study to select seeds of three varieties of *P. vulgaris* var. strike, contender, and wonder which had different seed coats colours (creamy, Brown to deep brown, and Reddish spotty (pinto) beans respectively). The study 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 current study also estimated amounts of anthocyanin and proanthocyanidins (PA) as pigments that give seed coats their brown color. The antioxidant potential of these seeds were also assessed based on free radical scavenging, 2, 2-diphenyl-1-picrylhydrazyl, β-carotene-linoleate, total phenolic, and flavonoid contents values. This to avail critical information in developing practical strategies to enhance *P. vulgaris* L.quality and market prizes from one hand and to correlated the genetic variations among three varieties with their antioxidant potential status from other hand.

In order to provide information on breeding and crop improvement; genetic variations among seeds of three varieties during different seed developmental stages which included (full dry seeds > dry and imbibed seed coats > dry and imbibed embryos > radical > and pollen grains) was assessed using two biomarkers. These biomarkers were protein-based biochemical marker using soduim dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and free amino acids using High-Performance Liquid Chromatography (HPLC-Analysis) in addition to molecular marker based on DNA analysis using random amplified polymorphic DNA (RAPD). These markers will give full identification of studied varieties germplasm for genetic conservation and ensuring their sustainable use.

**Phenotypic marker** based on phenotypic parameters of seed germination and seedling growth of three varieties. Seed germination parameters after 2, 4, 6 days represented in the final germination percent (FGP), means daily germination (MDG), mean germination time (MGT), coefficient of velocity of germination (CVG) while seedling growth parameters at different vegetative stages after 15 and 30 days represented

in mean lengths of root , shoot, and seedlings (cm), mean root /shoot ratios, dry weight of seedling , first seedling lengths vigour index (Vi) I, second dry seedling weight vigour index (Vi) II in addition to leaf parameters which represented in number of leaves per plant, and leaf surface area ($cm^2$).

Phenotypic features based on flower color and fruiting criteria; the current data showed considerable variations in the flower color of three *P. vulgaris* L. varieties that ranged from deep violet in contender variety to white - creamy and creamy-pale violet in

strike and wonder varieties respectively while their fruiting criteria showed variations in the mean values of pods number / plant, the mean number of seeds / pod, average weight of 100 seeds/g, and seed sizes measuring by this weight.

**Phenotypical marker** represented in macro and micro- phenotypical aspects which considered specific feature of seed coat of each *P. vulgaris* L. variety. Data obtained by stereomicroscope investigation based on macro -phenotypical aspects showed clear variations among three *P. vulgaris* varieties based on 9 criteria including shape, texture, phenotypic seed color, average seed sizes (mm) based on mean length and width of seeds in addition to hilum aspects based on position, shape and size (mm). On the other hand, data obtained by scanning electron microscope (SEM) investigation based on macro -phenotypical aspects showed clear variations among three *P. vulgaris* L. varieties based on 8 criteria including overall seed coat pattern, epidermal cell; anticlinal walls based on shape, thickness, texture and height; and periclinal walls based on surface, texture, average cell size (µm); each one emerged as a suitable character. Our observations showed that by using macro-phenotypical aspects, varieties will identify even if only seed characters were used.

**Anthocyanin, proanthocyanidins, and antioxidant potential assays** of seeds of three *P. vulgaris* L. varieties exhibited clear variations in contents of anthocyanins, proanthocyanidin and antioxidant activity. All varieties showed antioxidant activity but contender bean seeds with brown to deep brown seed coat color were consisted of greater amounts of anthocyanins and proanthocyanidin and exhibited superior antioxidant activity.

**Biochemical protein markers using [SDS-PAGE] technique** represented in seed storage proteins analysis of each variety during 7 different developmental stages by SDS-PAGE technique. SDS-PAGE analysis showed that all varieties and each variety at different developmental stages exhibited distinctive quantitative and qualitative alterations in protein electrophoretic patterns during each developmental stage. These protein alterations based on changes in number of polypeptides bands, their molecular weights (MWs), polypeptides bands intensities, fractionation of some bands, appearance of new bands (unique bands), and disappearance of some bands (polymorphic bands) that led to highly levels of protein polymorphisms.

**Biochemical analysis of free amino acids using HPLC,** proteins are composed of amino acids; they were analyzed using HPLC, which showed variations in the amount of free amino acids between three *P. vulgaris* L. varieties and between each variety at different developmental stages; these variations may reflect the environmental oxidative stress of their ecogeographical origins. HPLC analysis revealed the presences of 17 amino acids of variable content ranging from 26.24%-40.75%, 9 of which are essential amino acids in humans.

**Polymorphic DNA marker using RAPD- PCR fingerprinting:** RAPD-PCR analysis DNA exhibited distinctive qualitative and quantitative alterations in the RAPD profiles between 3 colored seeds of *P. vulgaris* L. varieties and between each variety at

different developmental stages based on the number of amplified gene products, the amplified DNA sizes, their intensities, and appearance or disappearance of DNA bands that led to generation highly levels of DNA polymorphism. Each band of amplified products corresponding to one gene had specific randomly sequences with variable size and intensity specialized for each variety.

The current study concluded that all markers used in present study 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. Each technique has its own advantages and limitations. Phenotypical and protein-based markers are dependent on the gene expression of DNA at exons (coding) region only and may be influenced by environmental conditions, tissue specificity, developmental stages and age. On the other hand, the DNA-based markers are dependent on coding (exons) and non-coding (intron) regions and are not governed by above external factors because any changes in them are due to natural mutation within the gene sequence during replication that, in turn, may be repaired. Thus, 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 by which the conservation of the studied plant can be made easy. The current study showed also that *P. vulgaris* var . contender with brown to deep brown seed coat color were more pronounced than the other two varieties in most analyses used in this study.

## DISCUSSION:

### (1) Phenotypic parameters 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.

### (2) Phenotypic parameters based on macro and micro -phenotypical characters of three *P. vulgaris* L. varieties:

The results obtained in this study showed that the micro-phenotypical characters of seed coat observed specificity in distinguish each variety of three *P. vulgaris* L. varieties used in the current study. The present data observed that all macro- phenotypical properties of three varieties were clear distinction while micro- phenotypical characters were specificity in distinguish each variety and highly specific for each variety especially overall seed coat pattern, epidermal cell, anticinal walls and periclinal walls, each one emerged as a suitable character for each variety alone.

**a. Anthocyanin, proanthocyanidins, antioxidant activity, and total polyphenols of seeds of three *P. vulgaris* L. varieties:**

The current study showed that the three *P. vulgaris* L. varieties exhibited variations in anthocyanins, proanthocyanidin and antioxidant activity. Data obtained in the current study showed that Brown to deep brown seeds of contender bean variety exhibited the greatest amounts of anthocyanins and proanthocyanidins than creamy and Reddish spotty (pinto) of strike and wonder beans varieties.

**b. Analyses of seed developmental growth stages of *P. vulgaris* L.:**

Developmental growth stages have been described at the organismal level for a variety of experimental models of *P. vulgaris* L. The analysis of *P. vulgaris* L. growth and development presented here provides a framework methodology for identifying and interpreting phenotypic differences in plant resulting from genetic variation and/or environmental stress.

The utility of this methodology is validated through the discovery of new growth and development phenotypes for mutants identified previously as having defects in specific biochemical pathways but with subtle or no phenotypes observed at the organismal level. The seed growth stages and data collection methodology presented in this study can serve as a powerful means to unify the collection of phenotypic data. Reporting the growth stage during which data were obtained can provide an explicit developmental context for comparative purposes and enhance the value of data for future investigations.

Electrophoretic protein polymorphisms using SDS-PAGE technique: The present study observed that protein banding patterns of three *P. vulgaris* L. varieties during different developmental stages (full dry seeds > dry and imbibed seed coats > dry and imbibed embryos > radical > and pollen grains revealed qualitative and quantitative variations in terms of band number, staining intensity, and molecular weights of polypeptides bands.

Free amino acid Analysis by HPLC:Amino acids analysis by HPLC showed variation in free amino acids amounts among the three *P. vulgaris* L. varieties during different seed developmental stages separately, reflecting the environmental oxidative stress of each ecogeographical site and origin. This may be interpreted on the basis of a protein consists of a string of amino acids, each one of which is coded for by a triplet of nucleic acids in the string of DNA constituting a gene. For the protein to have physiological activity, the identity of many of these amino acids is essential. Thus, a change any part of the gene that causes a replacement of any one of these amino acids will prevent the physiological activity of the protein. It is impossible to say from observing the phenotype, lack of physiological activity of the protein, what change in the genotype has occurred. This is the most common form of many-to-one mappings of genotype onto phenotype.

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.

## CONCLUSION:

**The current study concluded the following:**

The current study showed that the contender bean seeds with brown to deep brown seed coat color were more pronounced than the other two varieties as the following:

1. 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 indictore.

2. In addition, they recorded the pronounced flower color and fruiting criteria values higher than the other varieties.

3. Their seed coats were the most and highly specify in macro and micro- phenotypical aspects.

4. Their seeds consisting of greater anthocyanins and exhibited greater antioxidant activity; consequently, this variety will be valuable genetic resource with high antioxidant activity and strong treatment of the diseases resulting from the oxidation and deformation of basic macromolecules responsible for gene expression and synthesis of proteins.

- The study also showed intervarietal variations within varieties and within each variety at different development stages, as shown by total seed protein data. The electrophoretic descriptors of the seed proteins observed in this study can be used as effective technique in identification and differentiation of *P. vulgaris* L. varieties.

- The result of this study indicated that, 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.

- The current study concluded that genes, polypeptides, and free amino acids varied according to variety type and seed developmental stage; this confirms that each seed developmental stage had its own genes, proteins, and free amino acids required for metabolic pathways during this stage to complete seed growth and seed yield for improving crop productivity.

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**الملخص باللغة العربية**

نبات الفاصول من البقوليات ذات القيمة الغذائية العالية ويتميزبتشكيلة واسعة من البذور ذات أنماط و ألوان مختلفة تغطي سمات مظهرية أساسية تميز أصنافه المختلفة كما أن ألوان غطاء البذور محكوم بمجموعة من الجينات داخل أنسجتها تنظم ميارات التخليق الحيوي للفينولات و أصباغ الانثوسيانينات و التي ترتبط بدورها في إظهار ألوان غطاء البذور. لذا اعتمدت الدراسة الحالية على اختيار ٣ أصناف من بذور الفاصوليا و هي كونتيندرcontender ، و ستريكstrike ، و الوندر wonder المختلفين في لون غطاء البذرة ( كريمي، بني الى بني غامق، و أحمر منقط ) و تقييم الخصائص المظهرية لهم باستخدام دليل مظهري على أساس إنبات البذور و نمو البادرات من ناحية ودليل مظهري على أساس غطاء البذور باستخدام الستيريوميكروسكوب و الميكروسكوب الإلكتروني من ناحية أخرى ، كما تم تعيين الخصائص المظهرية على أساس لون الزهرة و المقاييس الإنتاجية للثمار الخضراء.

اظهرت الدراسة الحالية إختلافات ضمنية بين أصناف الفاصوليا الثلاث عند كل مرحلة من مراحل النمو المختلفة عن طريقبيانات بروتينات البذور الإجمالية المتحصل عليها في الدراسة كما أن وصف التفريد الكهربائي للأنماط البروتين يجعلها تستخدم كتقنية فعالة في تعريف و تميز الاصناف المختلفة لنبات الفاصوليا. اظهرت بيانات الدراسة الحالية أن تقنية التكبير العشوائي لقطع DNA متباينة الأشكال RAPD نجحت نجاح فائق في تقدير التباين الوراثي للمادة بين الأصناف الثلاثة و يؤكد هذا على فاعلية هذه التقنية و مصداقيتها في بنك الجينات لمعرفة و تميز الأصناف المختلفة لنبات الفاصوليا. استنتجت الدراسة الحالية أنكل صنف من أصناف نبات الفاصوليا يحتوي على جينات و بروتينات و أحماض أمينية تختلف طبقا لنوع الصنف و مرحلة نمو البذرة ، هذا يؤكد على أن كل مرحلة نمو لديها جينات و بروتينات و أحماض أمينية خاصة بها لإتمام المسارات الأيضية اللازمة لهذه المرحلة و ذلك لإستكمال نمو البذرة وصولا للإنتاجية من أجل تحسين إنتاجية المحصول.

**الكلمات المفتاحية :** التحليل الجيني - الجزيئي - معاطف البذور - حبوب اللقاح – نبات الفاصوليا.