Egypt. J. Bot. **Vol. 60**, No. 2. pp. 361-380 (2020)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Protein and DNA Polymorphisms among Three Colored Seeds of *Phaseolus vulgaris* L. Cultivars during Seven Subsequent Stages of their Development

Ekram A. Mohamed[(1)#] , Mona M. Elrashd[(2)]
[(1)]Plant Cytogenetic and Molecular Genetics, Botany and Microbiology Department, Faculty of Science, Zagazig University, Sharkia, Egypt, P.O. Box 44519, Zagazig 308213, Egypt; [(2)]Biology Department, Faculty of Science and Arts, Shaqra University, Riyadh, Saudi Arabia.

THIS STUDY aimed to evaluate genetic variations among germplasms of three colored seeds of *Phaseolus vulgaris* cultivars (creamish yellow "strike", deep brown "contender" and reddish spotty pinto "wounder") during seven subsequent stages of seed development ranging from dormant seed to pollen grains. The evaluation based on the analysis of the data obtained from electrophoretic banding patterns of protein and DNA separated from each of the three studied cultivars, by using two different bioassays; sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). These analyses revealed major distinctive genetic variations among the three cultivars qualitatively and quantitatively. Besides, there were also variations in the percentages of polymorphisms in the produced protein and DNA bands due to the differences among their size, number, intensity, types, and gain or loss others. The highest number of polypeptide bands and amplified DNA products was scored at the contender cultivar, while the lowest number was scored at the strike cultivar during all stages of seed development. Meanwhile, the highest total number of polypeptide bands (64) and amplified DNA bands (108) were scored at the seedling and vegetative stages, respectively. This study concluded that data generated by the used bioassays were nearly equivalent, but not identical. Thus, it is better to be combined for reliable estimates of genetic variations among the three studied cultivars during their development to earn valuable genetic resources exploited for breeding new cultivars.

Keywords: Legume seeds development, *Phaseolus vulgaris* colored seeds, RAPD-PCR, SDS-PAGE.

Introduction

Seeds are an important organ that represents the delivery system for the transfer of genetic materials from one generation to the next, through the sexual reproduction in vascular plant and is evolutionarily advantageous for human life, animal, and plant survival (Li et al., 2012). Seed development of angiosperms involving distinct coordinated growth for three seed components; seed coat, embryo and endosperm, which different genetically. These seeds store starch, lipids and proteins and acts as a source of nourishment during germination and early seedling development (Sreenivasulu & Wobus, 2013). Moreover, seed development involves highly dynamic metabolic processes of cell division, differentiation, growth, pattern formation and macromolecule production. This will provide discernment view about the complex system coordinating metabolism and plant development (Li et al., 2012). On the other hand, embryonic development initiates the storage accumulation of reserve macromolecules such as proteins. Dean et al. (2011) reported that

[#]Corresponding author email: ekram.esa@gmail.com
Received 15/9/2019; Accepted 11/12/2019
DOI: 10.21608/ejbo.2019.16954.1356
Edited by: Prof. Dr. Soliman A. Haroun, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt.

seed coat protects the embryo from the external environmental factors and control its development during seed dormancy and maturation. In addition, it is important for seed dispersal and hydration, also it provide an interesting developmental system for studying and representing excellent model system for cellular metabolic processes. The same authors also confirmed that many genes are specifically expressed within the seed coat tissues that produce proteins responsible for phenotypic changes of seed coat color and inducing differentiation of the ovule integuments into the seed coat.

Moreover, the male gametophyte (pollen grains) are highly reduced haploid male gametes that have brief free-living stage enriched with all nutrients that are necessary for plant growth, development, fertilization process and crop production (Borg et al., 2009). Twell et al. (2006) reported that advanced genomic and genetic technologies have fueled significant progress in understanding pollen grains development at the molecular level. Molecular marker based on analysis of pollen grains DNA has identified a large number of genes that are expressed during stamen and pollen grains development producing a cultivar of proteins, including signal transduction proteins, transcriptional regulators, regulators of protein degradation, and enzymes. This required for specifying stamen identity, regulating anther cell division and differentiation, controlling male meiosis, supporting pollen development, and promoting anther dehiscence (Borg et al., 2009). In 2013, Nakazawa et al. reported that DNA analysis of pollen grains is a possible technique for identifying the genes at the species level.

The study of Weber et al. (2005) mentioned that legume seed development is highly related to metabolism, transport of nutrients. It is also characterized by progressive differentiation of organs and tissues resulting in developmental gradients. The stages of seed development are well established in many legume species, in *Phaseolus vulgaris* (Coelho & Benedito, 2008). The common bean (*Phaseolus vulgaris* L.) is a highly valuable nutritious food legume and exhibits a wide cultivar of seed coat colors. It is a diploid crop (2n=22) belonging to Leguminosae, genus *Phaseolus* L which is widely distributed in all parts of the world (Bareke et al., 2018). It is the most commonly consumed annual leguminous food crop worldwide, and serves as a big dietary role. It supplies the plant with major protein sources, carbohydrates, essential elements and vitamins to both rural and urban areas (Margaret et al., 2014).

During subsequent seed development, seed storage proteins are typically degraded by proteases that convert the insoluble storage proteins into soluble peptides and then by hydrolases to generate free amino acids. These free amino acids are available for transport to the embryonic axis to support growth and as an energy source. Upon seed development, small peptides and amino acids generated by the cleavage of storage proteins that may remain in the storage tissue or translocate to subsequent stages of seed development (Kesari & Rangan, 2011).

In 2012, Li et al. reported that genetic and molecular analyses have identified critical players in the process of seed development in recent years. According to our knowledge there are no reports available concerned with the genetic variations of the banding patterns and profiling of electrophoretic protein and DNA during subsequent seed development of colored seeds P. vulgaris species. This attracted the attention of this study to evaluate these genetic variations during the subsequent stages of seed development of common bean.

Genetic variability among and within plant species usually resulted from variations in DNA sequences during the duplication process and influence of the environment. The assessment of genetic variability is routinely performed using various biomarkers such as phenotypically and biochemically storage proteins and DNA-based molecular markers, especially RAPD-PCR (Govindaraj et al., 2015). Analysis of genetic variations in the electrophoretic patterns of storage proteins is a useful method for establishing relationships among plant cultivars within a species. Storage proteins play a fundamental role in human nutrition, livestock feed, and plant reproduction. They accumulate during seed development within protein bodies as reserve material for germinating seedlings (Mouzo et al., 2018).

The study of EL-Atroush et al. (2015) reported that proteins are the primary products of gene expression which reflect the genetic structure of the organism. These proteins are separated and identified by sodium dodecyl

sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique into specific banding patterns that generating higher levels of genetic polymorphisms. This was based on differences in protein intensity, gaining and losing polypeptide bands among genotypes (Singh et al., 2017). The electrophoretic profile of SDS-protein detected apparent variations among the genotypes that were enough to differentiate among them. The high level of protein polymorphism could be attributed to the non-conservative nature of protein that it easily affected by the environmental conditions (Ahmed & Al-Sodany, 2019).

On the other hand, DNA-based biomarker based on the polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD) can assess genetic variations within and among plant germplasms, which do not suffer from either environmental influences or from plant organs and developmental stages (Ahmed & Fadl, 2015). It is technically simple, independent of any prior sequence information, has a very high genomic abundance and polymorphisms, requires minimal DNA amount without the requirement for cloning, sequencing, or any other form of the molecular characterization of the genome (Fang et al., 2016). Moreover, the study of Welsh & McClelland (1991) reported that the RAPD technique is a very sensitive method of screening nucleotide sequence polymorphisms that are randomly distributed throughout the genome. This was observed in both coding and non-coding regions, and single copy (unique) or repeated sequences. The high polymorphism generated by RAPD-PCR markers may arise due to changes in the nucleotide sequence or mutations in the genome loci. This made it possible to identify genetic variations among individual plants or rare or endemic species or cultivars (Mondini et al., 2009). These polymorphisms are present either at or among primer binding sites, that can be detected in the electrophoresis by confirming the presence or absence of specific bands.

The present study was designed to give full credibility and reliable knowledge about the effect of subsequent stages of seed developmental on banding patterns of electrophoretic protein and DNA of three colored seeds of *P. vulgaris* cultivars using SDS-PAGE and RAPD-PCR analyses and to provide valuable information about breeding and crop improvement of this important legume crop.

Materials and Methods

Plant material and experimental design

Plant materials

Three colored seeds of well-known commercial cultivars of *Phaseolus vulgaris* L. (cv. Super strike, cv. Early contender and cv. Kentucky Wonder) with creamish yellow, deep brown and reddish spotty (pinto) seed coats were used in this study. Their name, colors and origin listed in Table 1.

Experimental design

Viable and healthy seeds of each *P. vulgaris* cultivar were screened for uniformity size, cleaned and surface sterilized in a 1% v/v solution of sodium hypochlorite by gentle stirring for 10 min, then rinsed three times with deionized water to ensure surface sterility and then air dried well. Seven distinct stages of seed development (Table 2) were used in this study to follow the genetic variations of three colored seeds common bean cultivars using protein-based biochemical analysis by SDS-PAGE and DNA-based molecular analysis by RAPD-PCR of each common bean material specify for each stage.

Plantation of seeds of the studied common bean cultivars

Twenty surface-sterilized common bean seeds of each cultivar were immediately sown in rows in earthenware pots (30cm high x 20cm diameter) containing soil obtained from topsoil for plantation under greenhouse conditions until reached seedling stage (15 days), vegetative stage (35 days), and flowering stage (60 days Fig. 1).

Protein and genomic DNA analyses

SDS-PAGE and RAPD-PCR biomarkers were used to assess genetic variations of the storage proteins and genomic DNA respectively among the three studied colored seeds of common bean cultivars during seven stages of seed development ranging from 0 to 60 days (Tables 1 and 2). Protein and genomic DNA were extracted from different common bean plant material specific for each stage of seed development as following:

I- Stage DS; dormant seed (embryo and seed coat).
II- Stage DSC; dry seed coat only.
III- Stage ISC; imbibed seed coat after 24 hours of sowing seeds in distilled water.
IV- Stage IEM; imbibed embryonic tissues without seed coats.

V- Stage S; seedling (after germinating embryo for 15 days).
VI- Stage V; vegetative (leaves after 35 days of planting of seeds).
VII- Stage F; flowering stage (pollen grains, PGs after 60 days of planting of seeds).

TABLE 1. Labels, name, seed coat color and origin of P. vulgaris cultivars used in the present study.

Labels	P. *vulgaris* cultivars	Physical appearance of seed coat color	Origin
A	Super strike - Green bean plants	Creamish yellow	USA
B	Early contender - Bush bean plants	Deep brown	USA
C	Kentucky wonder - Pole beans plants	Reddish spotty (pinto)	Turkey

TABLE 2. The code numbers and names of the seven stages of seed development of P. vulgaris cultivars used in the present study.

	Stages of seed development						
No.	**I**	**II**	**III**	**VI**	**V**	**VI**	**VII**
Stages	Dormant seed (DS)	Dry Seed Coat (DSC)	Imbibed Seed Coat (ISC)	Imbibed Embryo (IEM)	Seedling (S)	Vegetative (V)	Pollen grains (PGs)

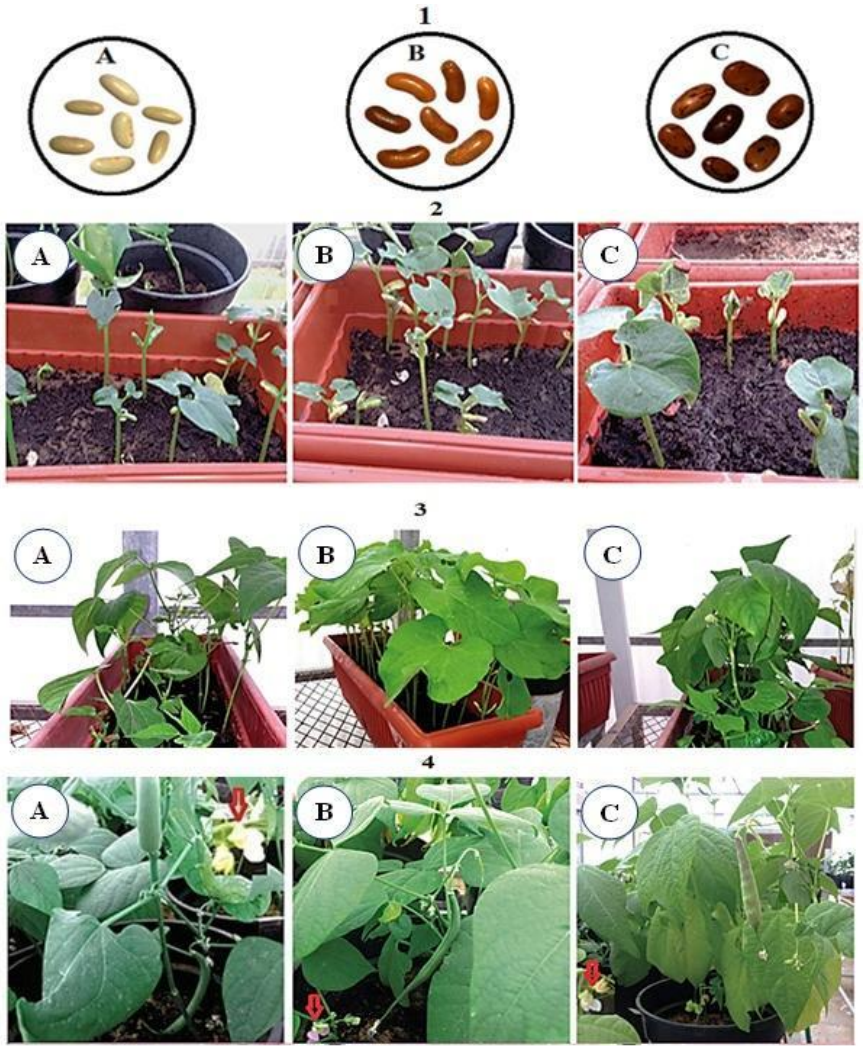

Fig. 1. Photographs illustrates different colored seeds of three *P. vulgaris* cultivars (A: Strike B: Contender C: Wonder) and their development; 1- dry seeds, 2- 15 days (seedling stage), 3- 35 days (vegetative stage), and 4- 60 days (flowering stages contained pollen grains as referred by the arrows) from planting of seeds.

Collection of pollen grains during flowering stage used in SDS-PAGE and RAPD-PCR techniques

Mature flowers of common bean cultivars were collected during the flowering stage. Anthers were separated from the flowers. The anthers were crushed gently after drying and pollen grains were sieved through (100, 200 and 300µm) mesh sized sieves. To remove lipids and irritants of low molecular mass, the pollen sample was defatted with diethyl ether by repeated changes, until the ether becomes colorless. Pollen purity was checked by light microscopy and then they were treated with acetone and dried. The defatted pollen grains powder was then completely vacuum-dried and stored at -4°C in airtight containers until further use as described by Hossain et al. (2012).

Protein analysis using SDS-PAGE technique

Cleaned and sterilized common bean plant materials specify for each stage were ground and defatted according to the methods described by Hojilla-Evangelista & Evangelista (2006).

Extraction of storage protein using SDS-PAGE technique

The protein extraction technique used was similar to that employed in the study of Abdelhaliem & Al-Huqail (2016) with some modifications. The sample buffer was added to 0.2g seed flour and mixed thoroughly in an Eppendorf tube by vortexing. The extraction buffer contained the following: 0.5M Tris-HCl, pH 6.8, 2.5%, SDS 5%, urea, and 5% 2 mercaptoethanol. Before centrifugation at 10,000g for 5min at 4°C, the sample buffer was boiled for 5 min. A standard method on a vertical slab gel was used for SDS-PAGE. Bromophenol blue was added to the supernatant as a tracking dye to visualize the movement of protein on the gel. Proteins in samples were profiled using SDS polyacrylamide gels as described by Laemmli (1970). Storage proteins were analyzed by SDS-PAGE using polyacrylamide gel (10%). Coomassie brilliant blue G-250 stain was used for visualizing the polypeptide bands on gel after electrophoresis. Marker proteins (Fermentas) were used as references. The polypeptide bands produced in the electropherogram were scored and their molecular weights were compared to the standard Pharmacia protein marker.

Electrophoretic protein imaging and data analysis

Gels for protein electrophoresis were photographed and documented using the Bio-Rad gel documentation system. The bands on each lane were counted and compared using Gel Pro-Analyzer software, while quantitative variations in band number, band types [polymorphic (unique and un-unique) and monomorphic bands], and band density were estimated using Bio-Rad Video densitometer, and Model Gel Doc 2000 system. To assess the variations in the protein banding pattern, electropherograms of each cultivar germplasm was scored for the presence or absence of polypeptide bands.

Genomic DNA extraction and analysis using RAPD-PCR technique

Extraction of genomic DNA from each plant material of common bean cultivar as well as quantity and quality of isolated DNA were carried out as the method described by Abdelhaliem & Al-Huqail (2016) with some modifications.

RAPD-PCR analysis and agarose gel electrophoresis

PCR amplification of purified genomic DNA using random RAPD primers and agarose gel electrophoresis were conducted as described in the study of Abdelhaliem & Al-Huqail (2016). A total of 20 random DNA oligonucleotide primers from Thermo Scientific (10mer) were independently selected to amplify the DNA samples in the PCR (UBC, University of British Columbia, Canada) following the method of (Williams et al., 1990) with some modifications. Only seven primers (P-01, 02, 03, 04, 05, 06 and 07) succeeded to generate reproducible amplified DNA products.

RAPD profiles and data analysis

Analysis of RAPD bands based on qualitative and quantitative variations (the number of amplified DNA bands, their size, intensity as well as the loss of normal DNA bands or gain of new ones were performed by Bio-One D++ Software (Vilber Lourmat, France). Data were scored as the presence or absence of DNA bands by making a binary matrix (1 if present and 0 if absent) for each cultivar at each developmental stage. Types of DNA Bands [Polymorphic DNA bands (unique and non-unique) and monomorphic bands] were also scored.

Estimation of Protein and DNA Polymorphisms

Protein or DNA polymorphism based on the loss of bands (non-unique) and the appearance of a new band (unique bands) as well as the monomorphic bands (bands with the same loci at all samples), were estimated by using the following equation:

$$\text{Protein or DNA Polymorphism \%} = \frac{\text{Polymorphic DNA bands (unique and non-unique)}}{\text{Polymorphic and monomorphic bands}} \times 100$$

Results

Protein and genomic DNA analyses

The current study used SDS-PAGE and RAPD-PCR techniques to assess polymorphisms of protein and DNA banding patterns respectively among three colored seeds of common bean cultivars during seven stages of their seed development (Tables 1 and 2) to assess genetic variations occurred during these stages that are required for vital metabolic processes taking place during each stage.

Survey of electrophoretic protein polymorphisms using SDS-PAGE technique

Polypeptide banding pattern and profile generated by SDS-PAGE analysis exhibited distinctive variations among three colored seed common bean cultivars and among stages of seed development based on band number, band intensity, molecular weight (kDa) of polypeptide bands and their fractionation, band type (polymorphic bands and monomorphic bands), appearance of new bands (unique loci), and absence of others (non-unique loci) as indicated in Table 3 and Fig. 2.

At the level of seed color, the maximum number of polypeptide bands scored at deep brown (contender) cultivar, while the minimum band number scored at creamish−yellow (strike) cultivar, during all the stages of seed development. On the other hand, seedling stage scored the highest number of polypeptide bands (64) with molecular weight ranged from 205.98 to 15.50kDa, while the lowest number of bands (48) scored at imbibed embryonic tissues with molecular weight ranged from 185.33 to 17.07kDa as shown in Table 3.

Unique bands (private allele) that appeared only at one locus at one cultivar can be used as markers for genetic variation among plant species and cultivars. Stage of dormant seed (DS) scored the highest number of unique bands (18) distributed between three colored seed common bean cultivars; creamish yellow bean strike cultivar scored the highest number of unique polypeptides bands (9 bands) with molecular weight (116.00-108.20-101.8-30.5-28.4-25.7-20.0-18.0-14.4 kDa), while the lowest number of unique bands (1) scored at creamish yellow bean strike cultivar during imbibed seed coat and flowering stages with molecular weight (30.8 and 151.15kDa) respectively. The unique bands were absent at strike and contender cultivars during vegetative stage.

The percentage of protein polymorphism varied among three *P. vulgaris* cultivars during stages of seed development depending on the presence and number of monomorphic bands (bands appeared at the same loci in three cultivars but differing in band intensity). The highest level of protein polymorphism (74.19%) scored at stage of dormant seed while the lowest level of protein polymorphism (41.67%) scored at vegetative stage which scored the highest number of monomorphic polypeptides bands (14 bands).

Genomic DNA analyses

Survey of DNA polymorphism using RAPD-PCR technique

Amplified DNA banding patterns generated by RAPD-PCR analysis were investigated to determine the genetic variations of amplified DNA products among three colored seed coats of *P. vulgaris* cultivars occurring during seven distinct stages of seed development (Tables 4, 5 and Fig. 3). The code and sequences of the seven RAPD primers (P-01, 02, 03, 04, 05, and 08) are listed in Table 4.

RAPD analysis revealed a distinctive electrophoretic DNA banding pattern and profile varied among common bean cultivars and stages of seed development based on number of amplified DNA bands, band intensity, amplicon lengths (bp) and their fractionation, band type [polymorphic bands (unique and non-unique), and monomorphic bands], appearance of new DNA bands (unique), and absence of other bands (non-unique). At the level of seed coat color, the highest number of amplified DNA products scored at deep brown (contender) cultivar, while the lowest number scored at creamish yellow (strike) cultivar during all stages of seed development. At the level of seed developmental stages, the maximum number of amplified DNA products (108 bands) with amplicon DNA lengths ranged from 961.50 to 233.80bp scored at vegetative stage, while the minimum band number (100 bands) scored at imbibed embryonic tissues with amplicon DNA lengths ranged from 948.70 to 290.80bp as shown in Table 4.

TABLE 3. Analyses of electrophoretic protein banding patterns obtained from the studied *P. vulgaris* cultivars during different developmental stages using Gel Pro-Analyzer Software and Bio-Rad Video Densitometer.

Seed stages	Lanes and cultivars	Polypeptide bands				Types and frequency of polypeptide bands						Percent of polymorphism
						Unique (U)		Polymorphic (U + Non-U)		Monomorphic		
		No.	Total	%	Mol. Weights (kDa)	No.	%	No.	%	No.	%	
Dormant seed (DS)	A	19.00	57.00	33.33	190.14–13.46	18.00	31.58	23.00	40.36	8.00	14.04	74.19
	B	22.00		28.07	190.14–15.82							
	C	16.00		38.60	190.14–16.30							
Dry seed coat (DSC)	A	21.00	60.00	35.00	199.03–14.40	15.00	25.00	18.00	30.00	8.00	13.33	69.23
	B	23.00		38.33	199.03–17.24							
	C	16.00		26.67	199.03–15.11							
Imbibed seed coat (ISC)	A	17.00	57.00	29.83	188.40–14.60	6.00	10.53	13.00	22.81	10.00	17.54	56.52
	B	21.00		36.84	188.40–12.63							
	C	19.00		33.33	188.40–13.52							
Imbibed Embryo (IEM)	A	14.00	48.00	29.17	185.33–17.51	12.00	25.00	17.00	35.42	8.00	16.67	68.00
	B	18.00		33.33	185.33–17.07							
	C	16.00		37.50	185.33–20.17							
Seedling (S)	A	20.00	64.00	32.81	205.98–17.68	15.00	23.44	20.00	31.25	9.00	14.06	68.97
	B	23.00		31.25	205.98–16.59							
	C	21.00		35.94	205.98–15.50							
Vegetative (V)	A	20.00	60.00	33.33	195.00-14.40	3.00	5.00	10.00	16.67	14.00	23.33	41.67
	B	21.00		35.00	195.00–14.40							
	C	19.00		31.67	195.00–14.40							
Pollen grains (PGs)	A	17.00	50.00	34.00	180.8–16.99	7.00	14.00	11.00	22.00	11.00	22.00	50.00
	B	18.00		30.00	180.8–16.99							
	C	15.00		36.00	180.8–16.99							

A = Strike, B = Contender, and C = Wonder cultivars.

TABLE 3 (Cont.). Analyses of electrophoretic protein banding patterns obtained from the studied *P. vulgaris* cultivars during different developmental stages using Gel Pro-Analyzer Software and Bio-Rad Video Densitometer.

Seed stages	Lanes	Unique polypeptide bands (U)			
		No.	Total	%	Mol. weights (kDa)
Dormant seed (DS)	A	9.00	18.00	41.19	116.00 –108.20–101.80–30.50–28.40–25.70– 20.00–8.00–14.40
	B	4.00		29.45	112.80–104.10--31.00–26.80
	C	5.00		29.45	109.70–103.90–42.00–38.00–29.80
Dry seed coat (DSC)	A	4.00	15.00	26.67	106.82–109.60–20.10–14.00
	B	6.00		40.00	199.50– 115.90–43.00–27.00– 26.25–17.95
	C	5.00		33.33	117.90–116.30–56.30–46.10–13.90
Imbibed seed coat (ISC)	A	3.00	6.00	50.00	45.00–15.00–14.60
	B	2.00		33.33	44.40–32.20
	C	1.00		16.67	30.80
Imbibed Embryo (IEM)	A	5.00	12.00	41.67	114.50–112.60–63.80–57.00–36.00
	B	4.00		33.33	99.50–60.60–46.30–23.25
	C	3.00		25.00	105.50–50.40–42.00
Seedling (S)	A	4.00	15.00	26.67	55.60–46.20–28.50–25.25
	B	6.00		40.00	158.13–130.60–123.50–65.70–64.60–45.00
	C	5.00		33.33	156.50–63.50–54.60–53.20–44.40
Vegetative (V)	A	0.00	3.00	0.00	0.00
	B	0.00		0.00	0.00
	C	3.00		100.00	101.17–49.04–46.01
Pollen grains (PGs)	A	1.00	7.00	14.29	151.15
	B	2.00		28.57	113.17–42.12
	C	4.00		57.14	108.19–49.24–41.00–32.50

A = Strike, B = Contender, and C = Wonder cultivars.

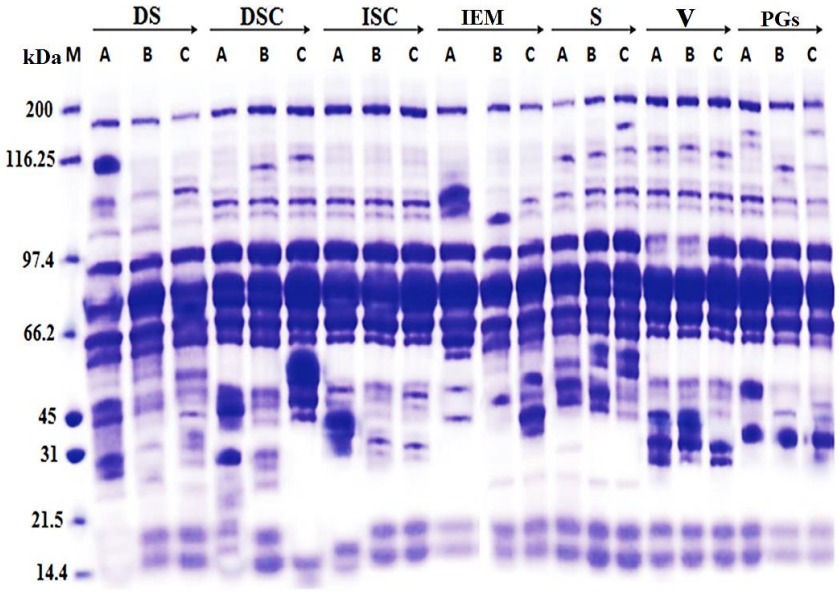

Fig. 2. Polypeptide banding patterns of the storage proteins extracted from the three studied *P. vulgaris* cultivars (A: Strike, B: Contender, and C: Wonder) during seven stages of seed development using SDS-PAGE techniques [M: is the standard protein marker. Descriptions of abbreviations of stages are listed in (Tables 1 and 2)].

TABLE 4. Codes and sequences of the seven RAPD primers and amplified DNA products generated by random amplified polymorphic DNA of genomic DNA extracted from the three studied *P. vulgaris* cultivars during seven seed developmental stages (Dormant seed, dry seed coat, and imbibed seed coat).

Primers code	Primers sequences (5′ → 3′)	Stages of seed development											
		Dormant seed (DS)				Dry seed coat (DSC)				Imbibed seed coat (ISC)			
		DNA size (bp)	# of DNA bands/ cultivar			DNA size (bp)	# of DNA bands / cultivar			DNA size (bp)	# of DNA bands / cultivar		
			A	B	C		A	B	C		A	B	C
P-01	CAA ACG TCG G	952.10-814.40	3	3	3	941.80-814.40	3	3	3	950.40-808.80	4	4	3
P-02	TCT GTG CTG G	880.70-815.30	3	3	2	886.70-810.90	2	2	2	884.30-808.70	2	2	2
P-03	GTG ACG TAG G	956.60-767.00	5	6	6	946.10-659.00	8	7	9	949.60-604.30	7	3	7
P-04	GGT CCC TGA C	816.40-393.70	5	4	4	859.50-363.40	5	4	3	850.30-335.50	6	7	6
P-05	AAT CGG GCT G	880.70-352.00	4	5	5	465.40-886.70	3	3	3	948.70-492.10	4	4	4
P-06	AGT CAG CCA C	959.80-788.50	8	7	5	951.20-587.30	6	9	6	941.10-672.40	4	8	5
P-07	TGC CGA GCT G	946.20-325.00	8	8	8	941.10-335.50	8	8	8	958.10-330.20	8	8	8
Total bands in each lane			34	36	33		35	36	34		35	36	35
Overall total bands				103				105				106	
% of bands			33.01	34.95	32.04		33.33	34.29	32.38		33.02	33.96	33.02

A = Strike, B = Contender, and C = Wonder cultivars.

TABLE 4 (Cont.) . Codes and sequences of the seven RAPD primers and amplified DNA products generated by random amplified polymorphic DNA of genomic DNA extracted from the three studied *P. vulgaris* cultivars during seven seed developmental stages (Imbibed embryo, seedling, vegetative, and pollen grains).

Primers codes	Stages of seed development														
	Imbibed Embryo (IEM)				Seedling (S)				Vegetative (V)				Pollen grains (PGs)		
	DNA size (bp)	# of DNA bands / cultivar			DNA size (bp)	# of DNA bands / cultivar			DNA size (bp)	# of DNA bands / cultivar			DNA size (bp)	# of DNA bands / cultivar	
		A	B	C		A	B	C		A	B	C		A	B	C
P-01	948.70-809.90	3	3	3	952.1-808.8	4	4	4	947.0-815.5	3	3	4	948.7-814.4	4	4	4
P-02	883.10-813.00	2	2	2	879.5-807.6	2	2	2	883.1-797.1	2	2	2	898.8-802.2	2	2	2
P-03	947.80-745.70	6	7	7	946.1-758.9	6	6	6	956.6-764.3	6	6	6	960.2-775.1	7	7	7
P-04	846.80-290.80	4	4	4	853.7-264.8	4	4	4	818.7-233.8	3	3	4	815.3-256.7	5	5	5
P-05	947.00-454.40	3	5	3	965.5-416.3	5	5	3	950.4-465.4	4	4	5	943.7-436.7	5	5	4
P-06	944.50-809.80	6	6	6	949.5-808.7	6	6	6	961.5-809.8	7	7	7	961.5-830.9	5	5	5
P-07	942.80-304.80	8	8	8	947.8-330.2	8	8	9	944.5-335.5	10	10	10	959.8-387.4	8	8	8
Total bands in each lane		33	35	32		34	35	35		35	38	35		35	36	36
Overall total bands			100				104				108				107	
% of bands		33.00	35.00	32.00		32.69	33.65	33.65		32.41	35.20	32.41		32.71	33.65	33.65

A = Strike, B = Contender, and C = Wonder cultivars.

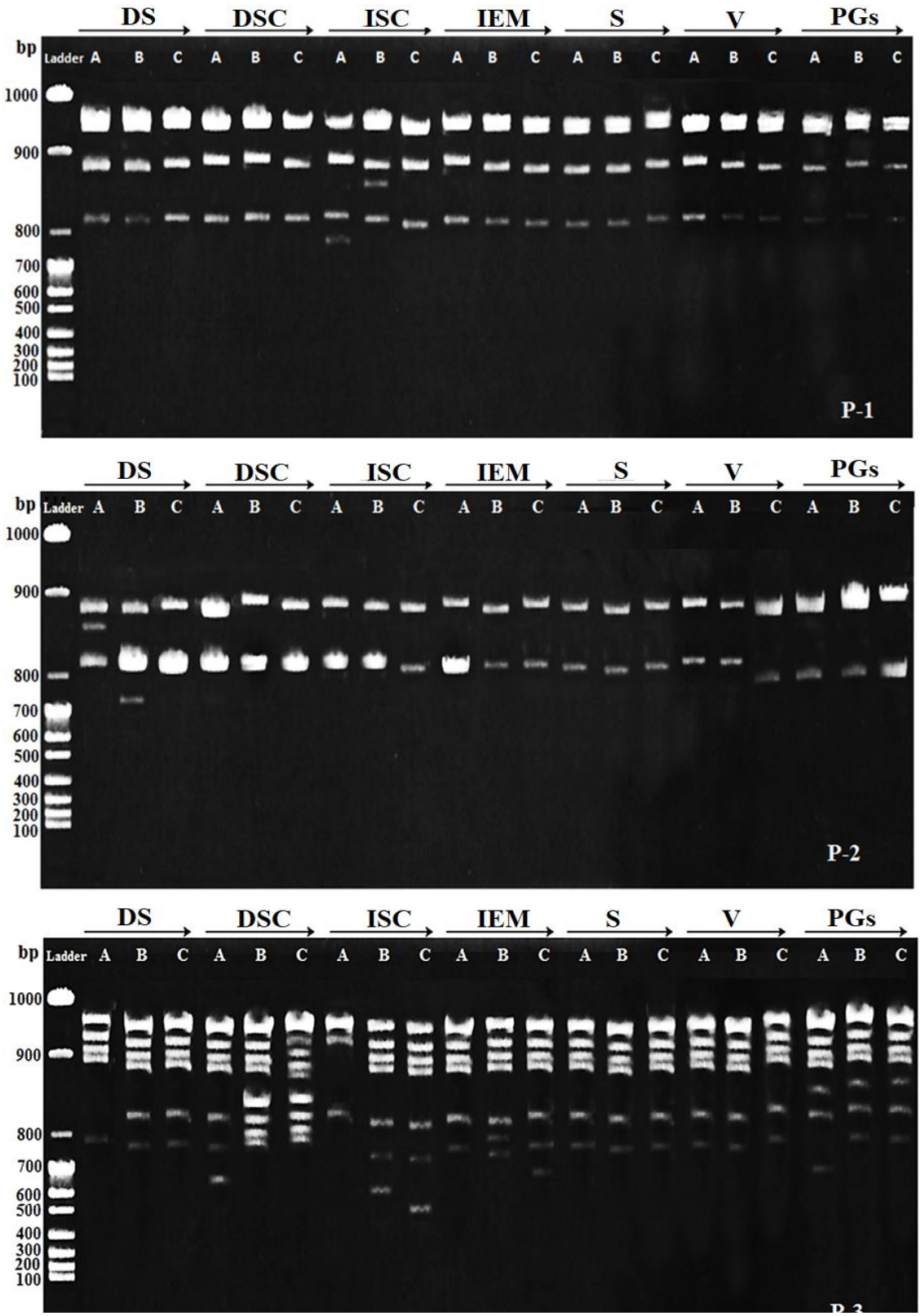

Fig. 3. DNA banding pattern generated by RAPD-PCR analysis of the genomic DNA extracted from the three studied *P. vulgaris* cultivars (A: Strike, B: Contender, and C: Wonder) using three random operon primers (P1-P3) during the seven seed developmental stages [DNA marker: 100-1000 bp ladder. Descriptions of abbreviations of stages are listed in (Table 2)].

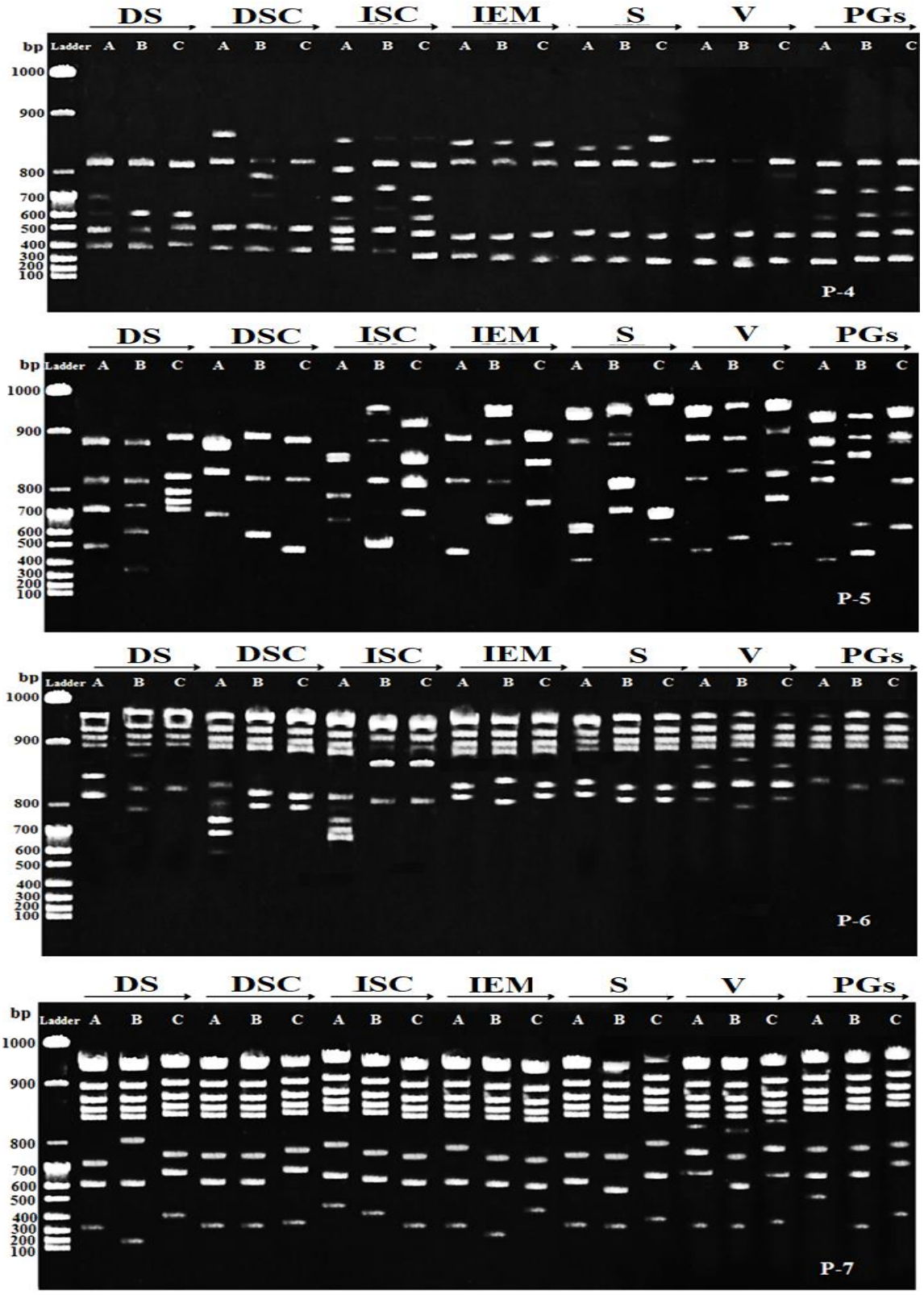

Fig. 3. (Cont.) DNA banding pattern generated by RAPD-PCR analysis of the genomic DNA extracted from the three studied *P. vulgaris* cultivars (A: Strike, B: Contender, and C: Wonder) using three random operon primers (P4-P7) during the seven seed developmental stages [DNA marker: 100-1000 bp ladder. Descriptions of abbreviations of stages are listed in (Table 2)].

TABLE 5. Number, types, and frequency of amplified DNA bands (unique, polymorphic, and monomorphic bands) of the three studied *P. vulgaris* cultivars during seed developmental stages using Bio-One D++ Software.

Dormant seed (DS)

Primer Codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	9	8.74	0	0	1	895	0	0	2	22.22	2	22.22	50
P-2	8	7.77	1	860	1	720	0	0	2	25	4	50	66.66
P-3	17	16.5	4	970-950-920-790	0	0	0	0	1	5.88	9	52.94	90
P-4	13	12.62	1	700	0	0	1	805	3	23.08	3	23.08	50
P-5	14	13.59	1	498	3	720-600-310	2	993-840	0	0	8	57.14	100
P-6	18	17.48	4	960-930-840-810	2	870-780	0	0	2	11.11	9	50	81.82
P-7	24	23.3	2	702-303	2	803-200	3	774-696-400	5	20.83	6	25	54.55

Dry seed coat (DSC)

Primer Codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of Polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	9	8.57	0	0	1	880	0	0	2	22.2	2	22.22	50
P-2	6	5.71	0	0	1	870	0	0	1	16.67	2	33.33	66.67
P-3	24	22.9	2	760-650	0	0	4	965-956-935-895	2	8.33	13	54.17	86.67
P-4	12	11.42	1	955	2	897-795	0	0	3	25	3	25	50
P-5	9	8.57	3	890-847-789	2	896-580	2	893-475	0	0	8	88.89	100
P-6	21	20	4	845-865-705-600	0	0	0	0	5	23.81	5	23.81	50
P-7	24	22.85	0	0	0	0	3	878-798-398	5	20.83	6	25	54.55

A = Strike, B = Contender, and C = Wonder cultivars.

TABLE 5 (Cont.) Number, types, and frequency of amplified DNA bands (unique, polymorphic, and monomorphic bands) of the three studied *P. vulgaris* cultivars during seed developmental stages using Bio-One D++ Software.

Imbibed seed coat (ISC)

Primer codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	11	10.19	1	870	1	850	1	810	1	9.09	5	81.82	83.3
P-2	6	5.56	0	0	1	865	0	0	1	16.67	2	33.33	66.67
P-3	17	15.74	3	940-845-620	1	590	0	0	1	5.88	9	52.94	90
P-4	19	17.59	2	803-450	2	760-650	2	500-350	1	5.26	11	57.89	91.67
P-5	12	11.11	3	874-792-688	3	960-888-478	2	940-665	0	0	10	83.33	100
P-6	17	16.04	5	897-830-730-7000-696	0	0	0	0	2	11.76	8	47.06	80
P-7	24	22.64	3	806-694-470	3	840-708-550	3	755-620-330	5	20.83	9	37.5	64.29

Imbibed embryo (IEM)

Primer codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	9	9	1	890	0	0	1	885	2	22.2	2	2.22	50
P-2	6	6	0	0	1	868	0	0	1	16.67	2	33.33	66.67
P-3	20	20	2	970-770	2	830-755	2	855-710	3	15	9	45	75
P-4	12	12	0	0	0	0	1	390	3	25	2	16.67	40
P-5	11	11	1	478	3	953-876-670	2	862-770	0	0	8	72.73	100
P-6	18	18	0	0	2	844-805	0	0	4	22.22	4	22.22	50
P-7	24	24	2	780-345	1	290	1	470	5	20.83	5	20.83	50

A = Strike, B = Contender, and C = Wonder cultivars.

TABLE 5 (Cont.) Number, types, and frequency of amplified DNA bands (unique, polymorphic, and monomorphic bands) of the three studied *P. vulgaris* cultivars during seed developmental stages using Bio-One D++ Software.

Seedling (S)

Primer codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	12	11.54	0	0	0	0	1	980	2	16.66	2	16.66	50
P-2	6	5.77	0	0	1	865	0	0	1	16.67	2	33.33	66.67
P-3	18	17.31	0	0	2	825-788	1	876	3	16.67	6	33.33	66.67
P-4	12	11.54	0	0	0	0	1	880	3	25	2	16.67	40
P-5	13	12.5	5	970-895-660-657-410	5	975-900-890-807-720	3	990-700-500	0	0	13	100	100
P-6	18	17.31	3	970-850-830	0	0	0	0	3	16.67	6	33.33	66.67
P-7	25	24.04	2	970-677	2	930-602	7	980-940-885-867-823-712-403	1	4	16	64	94.13

Vegetative (V)

Primer codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	10	9.26	1	875	0	0	0	0	2	20	2	20	50
P-2	6	5.56	0	0	0	0	1	800	1	16.67	2	33.33	66.67
P-3	18	18	0	0	0	0	5	975-955-893-848-768	1	5.56	10	55.56	90.91
P-4	10	9.26	0	0	0	0	2	878-400	2	2	3	30	60
P-5	13	12.04	3	905-782-390	2	838-607	4	905-844-715-475	0	0	11	92.31	100
P-6	21	19.44	0	0	2	870-760	0	0	5	23.81	4	19.05	44.44
P-7	30	27.78	3	866-801-706	3	863-799-616	3	882-818-408	5	16.67	11	36.67	68.75

A = Strike, B = Contender, and C = Wonder cultivars.

TABLE 5 (Cont.) Number, types, and frequency of amplified DNA bands (unique, polymorphic, and monomorphic bands) of the three studied *P. vulgaris* cultivars during seed developmental stages using Bio-One D++ Software.

Pollen grains (PGs)													
Primer codes	Total DNA bands		Unique bands						Monomorphic		Polymorphic		% of polymorphism
			A		B		C						
	No.	%	No.	Size	No.	Size	No.	Size	No.	%	No.	%	
P-1	12	11.21	0	0	1	990	0	0	2	16.66	2	16.67	66.66
P-2	6	5.61	1	872	1	883	1	895	1	16.67	3	50	75
P-3	21	95.23	7	950-930-915-900-885-820-710	0	0	0	0	0	0	14	66.67	100
P-4	15	14.02	2	630-390	0	0	1	740	2	13.33	6	40	75
P-5	14	13.08	3	870-850-790-390	3	870-670-402	1	682	1	7.14	9	64.29	90
P-6	15	14.02	0	0	1	865	0	0	4	26.67	2	13.33	33.33
P-7	24	22.43	2	815-590	2	856-392	3	852-774-436	5	20.833	8	33.33	61.54

A = Strike, B = Contender, and C = Wonder cultivars.

Total amplified DNA products, polymorphic DNA bands (unique and non-unique), amplified DNA monomorphic bands, and the percentage of amplified DNA polymorphism generated by seven RAPD primers are listed in Table 4.

The total amplified DNA bands generated by the seven used operon RAPD primers varied among the three *P. vulgaris* cultivars during the developmental stages of the seeds. The maximum value of total amplified DNA products (30 bands) generated by primer (P-07) at vegetative stages, while the minimum value (6 bands) generated by primer (P-02) at all stages of seed development except the full dry seed stage it was (8 bands).

Amplified DNA unique bands generated by the seven used primers varied among different colored seeds of *P. vulgaris* cultivars during the seven stages of seed development. Seven amplified DNA unique bands were generated by primers (P-03 and P-07) scored at creamish yellow (strike) cultivar with amplicon lengths (950-930-915-900-885-820-710 bp), and at reddish spotty (wonder) cultivar with amplicon lengths (980-940-885-867-823-712-403 bp), respectively this was during the reproductive and seedling stages, respectively (Table 5).

The percentage of amplified DNA polymorphism generated by seven RAPD primers varied also among the three colored seed coat *P. vulgaris* cultivars during seven seed developmental stages based on the absence or presence of monomorphic bands (Table 5). The highest level of amplified DNA polymorphisms (100%) were generated by primer (P-05) at all stages of seed development except reproductive stage generated by primer (P-03) due to the absence of monomorphic bands, while the lowest level of amplified DNA polymorphism (33.33%) generated by primers (P-02 and P-03) scored at seedling stage because number of monomorphic DNA bands were larger than polymorphic bands (Table 5).

Discussion

The current study observed distinct variations in the electrophoretic protein and DNA banding patterns among common bean cultivars of different seed colors and different stages of seed development using SDS-PAGE and RAPD-PCR analyses, respectively. The highest number of polypeptide bands and amplified DNA products were found at deep brown (contender) cultivar, while the lowest number recorded at the creamish yellow (strike) cultivar at all seed developmental stages. Meanwhile, 64 polypeptide bands and 108 amplified DNA products scored at seedling and vegetative stages, respectively. While the minimum number of 48 polypeptide bands and 100 amplified DNA products scored at imbibed embryonic stage. Each protein band is primary

end product of gene expression of active structural genes. The increasing in the number of polypeptide bands may be attributed to gene expression of some genes for synthesis of additional proteins necessary for the cellular metabolic processes that take place at each stage of seed development, while increasing in amplified DNA bands (genes) may result from increasing the number of base-pair sequences within the genome due to large addition of nitrogenous bases or insertion of the amplified regions at the genomic level (Mondini et al., 2009; Williams et al., 1990).

Protein and DNA banding patterns variations generated by SDS-PAGE and RAPD-PCR analyses may be due to major changes in physiological status, gene expression, and metabolic events that are specific for each stage of seed development and for each common bean cultivar due to its specific seed coat color. In this respect, Le et al. (2010) concluded that most active genes in seeds are shared by all stages of seed development, although significant quantitative changes in gene activity occur in each stage of seed development. These changes regulated by small gene set that is either specific for metabolic process occurring at this stage, or up-regulated with respect to creamish yellow (strike) cultivar with amplicon lengths (950-930-915-900-885-820-710bp), and at reddish spotty (wonder) active genes at other stages of seed development. Silveira et al. (2004) reported that seeds accumulate nitrogen in proteins during seed development; their synthesis rate is mainly controlled by the availability of free amino acids due to their addition or deletion from polypeptide chain.

The data of the present study showed the appearance of new polypeptide and amplified DNA bands and disappearance of others from one common bean cultivar (creamish yellow "strike") and during one stage of seed development (Imbibed Embryo). At this contest, protein bands disappearance may be due to the degradation of reserve proteins, which was necessary for metabolic process during stages of seed development or may be precursors for other proteins or enzymes. While the appearance of newly polypeptide bands at specific stage during seed development, may be due to the synthesis of other new proteins showing metabolic functions at this stage. This was in agreement with the findings of (Kesari & Rangan, 2011). The abundance of these proteins and their activities will define the basal requirement and protein dynamics during seed development (Li et al., 2012).

Besides, both the appearance and disappearance of polypeptide and amplified DNA bands in this study may resulted from changes in the structure of DNA (e.g. insertion or deletion of DNA fragments of various lengths, breaks and transpositions), which occurred during subsequent stages of seed development leading to changes in the amino acids that form these proteins, this was in concomitant with the results of Mondini et al. (2009). These lost and gained genes may lead to changes in the amino acids that form proteins during their gene expression (Kumar et al., 2009).

The variations in polypeptide and amplified DNA banding patterns found in the present study based on number, intensity and sizes of their bands, which lead to highly polypeptide and DNA polymorphisms. These reflect heritable changes in the nucleotide sequences, both in exons (coding) that containing DNA sequences which determine primarily the amino acid sequences of the proteins, and in introns (non-coding) regions. This matches with the findings of Welsh & McClelland (1991). Moreover, the variations in DNA polymorphism may resulted due to a wide range of variations; from single to many nitrogenous base pair change, structural alterations, copy number variations, and repeated sequences, which occur at different subsequent stages of seed development (Williams et al., 1990). Indeed, the non-coding region of DNA that do not code for proteins tend to have more polymorphisms (Fowler et al., 1988).

Finally, the current study observed that altered banding patterns of proteins and DNA at each stage of seed development may be due to production and elimination of genes during subsequent stages and consequently, active proteins that might serve as a monitoring mechanism over those intricate metabolic processes and energy production. The accumulation of these proteins during seed development may be used during rapid cell division and cell structure construction. This was in agreement with the findings of (Li et al., 2012).

Conclusions

Data obtained in this study demonstrated that each stage of seed development comprises a specific set of genes and active proteins that

were necessary for rapid and complicated metabolic changes occurring during that stage of the developing seed. The combination of SDS-PAGE and RAPD-PCR techniques in this study gave important genetic information and sufficient degree of genetic variations in protein and DNA among the germplasms of the three studied colored seeds of common bean cultivars. This occurred during subsequent stages of their seed development to improve the valuable genetic resources and help breeding new cultivars of this legume crop.

References

Abdelhaliem, E., Al-Huqail, A.A. (2016) Genetic linkage between protein and DNA polymorphisms and antioxidant capacity of *Cuminum cyminum* L. accessions. *Genetics and Molecular Research,* **15**(4), gmr.15048916.

Ahmed, S.M., Fadl, M.A. (2015) RAPD based genetic diversity analysis within the genus Solanum. *Egyptian Journal of Botany,* **55** (2), 175 -185.

Ahmed, S.M., Al-Sodany, Y.M. (2019) Authentication of ecological, biochemical and molecular features for some Lamiaceae species from Saudi Arabia. *Egyptian Journal of Botany,* **59**(3), 1-14.

Bareke, T., Asfaw, Z., Woldu, Z., Medvecky, B., Amssalu, B. (2018) Diversity of common bean (*Phaseolus vulgaris* L., Fabaceae) landraces in South Eastern Ethiopia. *Advances in Plants & Agriculture Research,* **8**, 449-457.

Borg, M., Brownfield, L., Twell, D. (2009) Male gametophyte development: a molecular perspective. *Journal of Experimental Botany,* **60**(5), 1465-1478.

Coelho, C.M.M., Benedito, V.A. (2008) Seed development and reserve compound accumulation in common bean (*Phaseolus vulgaris* L.).*Seed Science and Technology,* **2,** 42-52.

Dean, G., Cao, Y.G., Xiang, D., Provart, N.J., Ramsay, L., Ahad, A., White, R., Selvaraj, G., Datla, R., Haughn, G. (2011) Analysis of gene expression patterns during seed coat development in Arabidopsis. *Molecular Plant,* **4,** 1074-1091.

EL-Atroush, H.A., EL-Shabasy , E., Tantawy, M.A., Barakat, H.M.S. (2015) Pollen morphology and protein pattern of *Nitraria retusa* and some selected taxa of Zygophyllaceae in Egypt. *Egyptian Journal of Botany,* **55**(2), 207-230.

Fang, J., Zhu, X. , Wang, C., Shangguan, L. (2016) Applications of DNA Technologies in Agriculture. *Current Genomics,* **17**(4), 379-386.

Fowler, J.C.S., Burgoyne, L.A., Scott, A.C., Harding, H.W.J. (1988) Repetitive deoxyribonucleic acid DNA and human genome variation— A concise review relevant to forensic biology. *Journal of Forensic Science*, **33,** 1111-1126.

Govindaraj, M., Vetriventhan, M., Srinivasan, M. (2015) Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International,* **2015**, 431487 (14 pages).

Hojilla-Evangelista, M.P., Evangelista, R.L. (2006) Effects of cooking and screw- pressing on functional properties of Cuphea PSR23 seed proteins. *Journal of the American Oil Chemists' Society,* **83,** 713-718.

Hossain, I., Talukder, G., Roy, N., Shaha, R.K. (2012) Analysis of the free amino acid content in pollen of Bangladeshi common fruits flower of known allergenic activity. *Agricultural Science Research Journal,* **2**(3), 11-16.

Kesari, V., Rangan, L. (2011) Coordinated changes in storage proteins during development and germination of elite seeds of *Pongamia pinnata*, a versatile biodiesel legume. *AoB Plants*, **2011**, plr026.

Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R., Pandey, B.K. (2009) Potential of molecular markers in plant biotechnology. *Plant Omics,* **2,** 141-162.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227,** 680-685.

Le, B.H., Cheng, C., Bui, A.Q., Wagmaister, J.A., Henry, K.F., Pelletier, J., Kwong, L., Belmonte, M., Kirkbride, R., Horvath, S., Drews, G.N., Fischer, R.L., Okamuro, J.K., Harada, J.J., Goldberg R.B. (2010) Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. *PNAS*, **107,** 8063-8070.

Li. W., Gao, Y., Xu, H., Zhang, Y., Wang, J. (2012) A proteomic analysis of seed development in *Brassica campestri* L. *PLoS ONE*, **7**(11), e50290.

Margaret, N., Tenywa, J.S., Otabbong, E., Mubiru, D.N., Ali, T. (2014) Development of common bean (*Phaseolus vulgaris* L.) production under low soil phosphorus and drought in Sub-Saharan Africa: A Review. *Journal of Sustainable Development,* **7**, 128-139.

Mondini, L., Noorani, A., Pagnotta, M.A. (2009) Assessing plant genetic diversity by molecular tools. *Diversity,* **1**, 19-35.

Mouzo, D., Bernal, J., López-Pedrouso, M., Franco, D., Zapata, C. (2018) Advances in the biology of seed and vegetative storage proteins based on two-dimensional electrophoresis coupled to mass spectrometry. *Molecules*, **23**, 2462.

Nakazawa, F., Uetake, J., Suyama, Y. , Kaneko, R., Takeuchi, N., Fujita, K., Motoyama, , H., Imura, S., Kanda, H. (2013) DNA analysis for section identification of individual Pinus pollen grains from Belukha glacier, Altai Mountains, Russia. *Environmental Research Letters*, **8**(1), 014032 (8pp).

Silveira, V., Balbuena, T. S., Santa-Catarina, C. (2004) Biochemical changes during seed development in *Pinus taeda* L. *Plant Growth Regulation,* **44**, 147-156.

Singh, B. K., Singh, A. K., Hotti, A. H., Kumar, J., Singh, S. K. (2017) Diversity analysis through SDS-PAGE of seed storage protein of pea genotypes. *Research in Environment and Life Sciences,* **10**, 449-452.

Sreenivasulu, N., Wobus, U. (2013) Seed-development programs: a systems biology-based comparison between dicots and monocots. *Annual Review of Plant Biology,* **64**, 189-217.

Twell, D., Oh, S.A., Honys D. (2006) Pollen development, a genetic and transcriptomic view. In: "*Plant Cell Monographs*", pp. 15-45. Vol. 3. The Pollen Tube.

Weber, H., Borisjuk, L.,Wobus, U. (2005) Molecular physiology of legume seed development. *Annual Review of Plant Biology,* **56**, 253-279.

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

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research,* **18,** 6531-6535.

تعَدُد أنماط البروتين والحمض النووي فيما بين ثلاثة ألوان من بذور نبات الفاصولياء الشائعة خلال سبع مراحل متتابعة من تطورها

إكرام عبدالحليم محمد[1]، منى مصلح الراشد[2]
[1] وراثة خلوية وجزيئية للنبات - قسم النبات والميكروبيولوجي - كلية العلوم - جامعة الزقازيق - الشرقية - مصر، [2] قسم البيولوجي - كلية العلوم والآداب - جامعة شقراء - الرياض - المملكة العربية السعودية.

الهدف الرئيسي لهذه الدراسة هو تقييم الإختلافات في الأصول الوراثية بين بذور ثلاث أصناف من الفاصولياء الشائعة (*Phaseolus vulgaris* L.) ذات ثلاثة ألوان مختلفة (كريمي مائل للصفرة، وبني غامق، وبني محمر منقط)، من خلال تتبع سبعة مراحل مختلفة من تطور البذرة (مرحلة البذرة الكامنة إلى مرحلة الإزهار). وذلك عن طريق تحليل المعلومات التي تم الحصول عليها من أنماط شرائط البروتين والحامض النووي الديوكسي ريبوزي (DNA) لكل من الأصناف الثلاثة على حدا، باستخدام نوعين من الدلائل الجزيئية (دلائل البروتين بواسطة تقنية التفريد الكهربائي SDS - PAGE، والدليل الجزيئي للتكبير العشوائي لقطع DNA بواسطة جهاز تفاعل البلمرة المتسلسل RAPD-PCR).

ولقد كشفت التحاليل الناتجة من هذه التقنيات عن وجود تغيرات وراثية مميزة في أنماط البروتين والـ DNA كمياً وكيفياً فيما بين الأصناف الثلاثة. وأيضاً إختلافات في نسبة التباين (Polymorphisms)الناتجة عن إختلاف في عدد وحجم وكثافة ونوع حِزم البروتين وحِزم الـ DNA وكذلك فقدها واكتسابها. ولقد سجل الصنف ذو لون البذرة البني الغامق أعلى عدد من حِزم عديدة البيبتيدات وحِزم الـ DNA أثناء مراحل الإنبات السبعة بينما سجل أقل عدد قد في الصنف ذو لون البذرة الكريمي المائل للصفرة. وفي نفس الوقت سُجِل أعلى عدد كلي (64) من الحِزَم البروتينية و(108) من حِزم الـ DNA عند مرحلة البادرة والمرحلة الخضرية على التوالي. واستخلصت الدراسة من البيانات الناتجة عن إستخدام تقنيات الدلائل الجزيئية أنها ذات دلائل متكافئة تقريبًا في التقييم لكنها غير متطابقة. وعلية فمن الأفضل دمجهم مع بعضهم البعض لإظهار التقييم الحقيقي والموثق للإختلافات الوراثية فيما بين الثلاثة أصناف محل الدراسة ومراحل تطور بذورها، للحصول على مصادر وراثية قيمة تُستَغل لتحسين تربية وإكثار أنواع جديدة.