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Genetic Relationships and Diversity among Pea (*Pisum sativum* L.) Genotypes Assessed using Agro-Morphological and Molecular Markers

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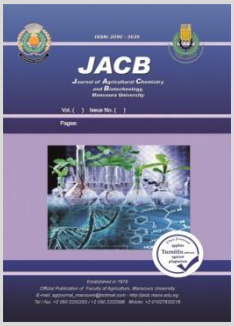

ABSTRACT

Fifteen pea (*Pisum sativum* L.) genotypes were evaluated for their agro-morphological performance and molecular diversity during 2017-2018 and 2018-2019 seasons. Combined analysis of variance revealed that there are highly significant differences among pea genotypes tested for all agro-morphological traits studied. The phenotypic coefficients of variation (PCV) values were relatively greater than Genotypic coefficients of variation (GCV) for all traits; however, GCV values were near to PCV values for the traits, like plant height, pods/plant, 100 seeds weight and pod width. High heritability coupled with high genetic advance was obtained for, plant height, reflecting the presence of additive gene action for the expression of this trait. High to moderate heritability coupled with moderate genetic gain were exhibited for number of pods per plant, 100 seeds weight, pod length, seeds per pod, pod weight and number of branches per plant. The Euclidean Distance among all genotypes based on agro-morphological traits was relatively wide. ISSR and SRAP markers were used to study the molecular diversity among pea genotypes. The molecular marker parameters revealed that SRAP markers were more efficient than ISSR markers with regards to polymorphism detection and in distinction among pea genotypes. Also, both ISSR and SRAP markers were able to amplify unique bands specific to a particular genotype. A positive and significant correlation ($r=0.389$: $p \leq 0.001$) between ISSR and SRAP matrix was observed according to Mantel's test. Also, significant correlation ($r = 0.411$: $p \leq 0.001$) between the matrices of combined molecular markers data and agro-morphological data.

Keywords: Pea, variability, heritability, genetic advance, ISSR, SRAP.

INTRODUCTION

Pea (*Pisum sativum* L.; 2n= 14) is an herbaceous annual in the Fabaceae (Leguminosae) family, and one of the oldest cultivated plants. It is a cool-season crop grown as a vegetable crop for both fresh and dried seeds in many parts of the world. In Egypt, early finds of peas date from c. 4800–4400 BC in the Nile delta area, and from c. 3800–3600 BC in Upper Egypt. Pea is widely cultivated for use in both animal feed and human food. Pea seeds contain relatively high proteins, carbohydrates, vitamins A, antioxidants, amino acids and minerals (Dahl *et al.*, 2012). Pea improvement through suitable breeding programs is the main option for the breeder to increase production and productivity. The efficiency of breeding methods depends on the availability of genetic diversity for that crop (Singh *et al.*, 2011).

Determining variability in agro-morphological traits of different pea genotypes will enable a breeder to know to what extent the environment affects yield and its component (Ullah *et al.*, 2012). Selection for higher yield only could be misleading because many factors interact to determine crop yield (Gatti *et al.*, 2005). Environmental factors are more effects on yield than their components, so selection for these components can be helpful to obtain genotypes with superior yield abilities (Gatti *et al.*, 2005). Genotypic coefficient variation, phenotypic coefficient variation and heritability proffer indication for genetic control for the expression of an assigned trait and phenotypic accuracy to prophesy its breeding value (Ullah *et al.*, 2012; Gupta *et al.*, 2020).

Cluster analysis category the genotypes into different groups based on Euclidian distance and give chance to select genotype that could produce superior hybrids (Subramanian and Subbaraman, 2010).

Different techniques such as agro-morphological, physiological, biochemical and molecular markers were used to study the genetic variations between and within pea genotypes. (Hoey *et al.*, 1996; Baranger *et al.*, 2004; Kwon *et al.*, 2012). Several studies have been used phenotypical characteristics and agronomical traits to determine the genetic diversity in the pea genotypes (Yirga *et al.*, 2013; Gixhari *et al.*, 2014; Ouafi *et al.*, 2016). However, Agro-morphological, physiological and biochemical markers are influenced by environmental conditions and do not necessarily show the genetic background; thence, molecular markers are required. .Molecular markers can overcome the difficulty over morphological markers in analyzing genetic divergence. These markers are sufficient in numbers, independent of tissue or environmental effects, high levels of detectable polymorphism and allow cultivar identification at an early development stage (Bebeli and Kaltsikes, 1993).

Many studies in peas have been conducted on assessment of genetic diversity among genotypes of different regions using several DNA markers, such as restriction fragment length polymorphism (RFLP; Dirlewanger *et al.*, 1994), random amplified polymorphism DNA (RAPD; Thakur *et al.*, 2018), simple sequence repeats (SSR; Mohamed *et al.*, 2019; Tahir *et al.*, 2018), sequence-related amplified polymorphism (SRAP; Esposito *et al.*, 2007;

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Guindon *et al.*, 2016 & 2019), inter-simple sequence repeat (ISSR; La´zaro and Aguinagalde, 2006; Pakseresht *et al.*, 2013) and amplified fragment length polymorphism (AFLP; Dyachenko *et al.*, 2014). Among them, SRAP and ISSR have gained popularity because they are simple, speed, effective, reproducible, and do not need prior information about the target sequences in the genome (Snowdon and Friedt, 2004).

Both ISSR and SRAP markers work in a different way within the genome, where ISSR marker targets simple sequences repeats in the genome (Zietkiewicz *et al.*, 1994) while SRAP marker target the open reading frame sequences Li and Quiros (2001).

In this study, to avoid the deviation resulting from the use of a single molecular marker, we further analyzed the genetic relationships of pea genotypes at the molecular level using ISSR and SRAP markers combined analysis to assess the genetic diversity of pea genotypes.

The objective of this investigation was to study the genetic behavior of some agro-morphological traits and estimate genetic distance among 15 pea genotypes, using agro-morphological traits and ISSR and SRAP markers. This would aid the long-term objective of identifying diverse parental lines to generate segregating populations for tagging important traits, with molecular markers.

MATERIALS AND METHODS

Field experiment of the present investigation was conducted in the Vegetable Experimental Research farm and molecular analysis was carried out at Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt, during two successive years (October, 2017/2018 and October, 2018/2019) to evaluate the performance of 15 pea (*Pisum sativum* L.) genotypes which namely; Balmoral (P1), Dwarf Gray Sugar (P2), Little Marvel (P3), Master B (P4), Jacuar (P5), Progress No.9 (P6), Entesar 1 (P7), Meteor (P8), Early Perfection (P9), Cash (P10), Digrass (P11), Lincoln (P12), Victory Freezer (P13), Alaska (P14) and Deltafon (P15).

Agro-morphological analysis

The experiment was carried out in a randomized complete block design (RCBD) with three replicates. Each genotype was depicted in each replicate by one row of 15 plants with rows set 60 cm from each other. Data were recorded on 10 plants in each row. The studied characters were: (1) date to flowering, (2) plant height, (3) pod length, (4) pod weight, (5) number of pods/plant, (6) number of seeds/pod, (7) 100 seeds weight, (8) number of branches/plant and (9) pod width.

Statistical and genetic analysis

Analysis of variance was performed by computer using MSTAT-C software program. ANOVA analysis was carried out according to Steel and Torrie (1980).

The genotypic and phenotypic variances (δ2g and δ2p) as well as Genotype x Year' interaction (δ2gy) are calculated as follow: δ2g = (MSG – MSE)/yr, δ2gy = (MSGY – MSE)/r and δ2p = δ2g + (δ2gy / r) + (δ2e / ry), Where MSG is mean square of genotype, MSE is mean square of the error, δ2e (environmental variance) = ME and r is number of replications. Moreover, genetic parameters, Genotypic and phenotypic coefficients of variation (GCV and PCV) are assessed following the method adopted by Kang *et al.* (1983). PCV (%) = (δp / $\bar{x}$) x 100 and GCV (%) = (δg / $\bar{x}$) x 100, Where: δp and δg are the phenotypic and genotypic standard deviation of the genotypes, respectively, and $\bar{x}$ is a genotypes mean of given trait. GCV% and PCV% values were categorized as low (0-10%), moderate (10-20%) and high (20% and above) as described by Sivasubramanian and Madhava Menon (1973). Heritability in broad sense was calculated according to Falconer (1989) as follows: h2b= (δ2g / δ2p) x 100. The heritability percentage was categorized as follows (0-30%), moderate (30-60%), and high (≥60%) as described by Robinson *et al.* (1949). Predicted genetic advance GA% was calculated using the method of Oladosu *et al.* (2014) as follows: GA%= K x δ2p x h2b x100, where K is selection intensity which assumed 5% and its value is 2.06. It was categorized as low (0-10%), moderate (10-20%), and high (≥20%). Euclidian distance and cluster analysis of agro-morphological traits were carried out using NTSYS-pc ver. 2.1 (Rolhf, 2000).

Molecular analysis

Total DNA was extracted from fresh leaves of 15 pea genotypes (10 leaves from random seedling as a bulk/genotype) using CTAB method (Murray and Thompson, 1980). with minor modifications of the extraction buffer. Quality and quantity of isolated DNA were checked by agarose gel electrophoresis and spectrophotometer.

Two molecular marker systems, 20 ISSR primers and 18 combinations of SRAP primers were used to screen the genetic diversity among 15 pea genotypes at molecular level. of all primers or primer combinations, 10 ISSR primers and 10 combinations of SRAP primers which gave consistently reproducible and polymorphic amplicons were select (Table 1).

PCR reactions were performed in a 25 µl volume, containing 50 ng of DNA, 1x PCR Buffer, 2 mM MgCl2, 200 µM each of dATP, dTTP, dGTP and dCTP, 2 µM of primer for ISSR and 1 µM of each of forward and reverse primer for SRAP and 1.2U Taq DNA polymerase. All of the components for PCR reaction were obtained from the company of Promega (http://www.promega.com). DNA amplification was carried out using SensoQuest, Lab-Cycler (Germany) with the following PCR program; For ISSR, initial denaturation at 94°C for 5 min, followed by 45 cycles each consisting of a denaturation step (94°C for 1 min), annealing step (annealing primer temperatures for 1 min) and extension step (72°C for 2 min) followed by a final extension (72°C for 10 min). For SRAP: initial denaturation at 94°C for 5 min followed by 10 cycles comprised of 1 min (94°C), 1 min (35°C) and 2 min (72°C), then 35 cycles of 1 min (94°C), 1 min (50°C) and 2 min (72°C), finally 10-min at 72°C. Amplified DNA amplicons were separated on agarose gel 2% (ISSR) and 2.5% (SRAP) in TBE 1X buffer, then stained with ethidium bromide and photographed using GelDoc-It ®2 Imager.

Data analysis

Amplified DNA amplicons using ISSR and SRAP markers were classified as absent (0) or present (1). Initially, total number of bands (TB), polymorphic bands (PB) and percentage polymorphism (PPB) were calculated. Further, distinguished power of each molecular marker was assessed using three parameters; polymorphic information content (PIC), marker index (MI) and resolving power (RP). PIC values were calculated according (Ghislain *et al.*, 1999); PIC

= [1 – (p2 + q2)], Marker index (MI) was calculated as MI = [PIC × ηβ] (Powell *et al*., 1996). Resolving power (RP) was calculated as RP = [∑Ib] (Prevost and Wilkinson, 1999).

Dice coefficient (Dice, 1945) used to estimate the genetic similarity, and the cluster analysis was performed using NTSYS-pc ver. 2.1 (Rolhf, 2000). The correlation between the distance matrices of ISSR and SRAP data, also between agro-morphological traits and combined data of ISSR and SRAP markers were estimated by Mantel-test (Mantel, 1967).

Table 1. ISSR and SRAP primer sequences

		Sequence (5′ to 3′)		
HB06		5'-GACAGACAGACAGACA-3'		
HB07		5'-ACACACACACACACACT-3'		
HB09		5'-GTGTGTGTGTGTGC-3'		
HB10		5'-GAG AGA GAG AGA CC-3'		
HB11		5'-GTG TGT GTG TGT TGT CC-3'		
UBC820		5'-GTGTGTGTGTGTGTGTC-3'		
UBC812		5'-GAGAGAGAGAGAGAGAA-3'		
UBC814		5'-CTCTCTCTCTCTCTCTA-3'		
UBC818		5'-CACACACACACACACAG-3'		
UBC844		5'-CTCTCTCTCTCTCTCTRC-3'		
SRAP -1	Me-1	5´-TGAGTCCAAACCGGATA-3´	Em-1	5´-GACTGCGTACGAATTAAT-3´
SRAP -2	Me-2	5´-TGAGTCCAAACCGGAGC-3´	Em-2	5´-GACTGCGTACGAATTTGC-3´
SRAP -3	Me-3	5´-TGAGTCCAAACCGGAAT-3´	Em-3	5´-GACTGCGTACGAATTGAC-3´
SRAP -4	Me-4	5´-TGAGTCCAAACCGGACC-3´	Em-4	5´-GACTGCGTACGAATTTGA-3´
SRAP -5	Me-5	5´-TGAGTCCAAACCGGAAG-3´	Em-5	5´-GACTGCGTACGAATTAAC-3´
SRAP -6	Me-6	5´-TGAGTCCAAACCGGACA-3´	Em-6	5´-GACTGCGTACGAATTGCA-3´
SRAP -7	Me-7	5´-TGAGTCCAAACCGGACG-3´	Em-7	5´-GACTGCGTACGAATTCAA-3´
SRAP -8	Me-8	5´-TGAGTCCAAACCGGACT-3´	Em-8	5´-GACTGCGTACGAATTCAC-3´
SRAP -9	Me-9	5´-TGAGTCCAAACCGGAGG-3´	Em-9	5´-GACTGCGTACGAATTCAG-3´
SRAP -10	Me-10	5´-TGAGTCCAAACCGGAAA-3´	Em-10	5´-GACTGCGTACGAATTCAT-3´

RESULTS AND DISCUSSION

Results

Genotype Performance and Analysis of Variance:

The combined analysis of variance showed that the mean squares of years, genotypes and their interaction were highly significant (P<0.01) in all studied traits, except year of number of branches (Table 2**).**

Overall the two years, data presented in Table (3) showed that the highest mean value for plant height (119.56 cm) was observed in the genotype P_{11}, while the lowest mean value (37.63 cm) was recorded for P_6 genotype. The genotype P_{14} revealed the maximum flowering days (77.16 day), and the minimum flowering days (39.33 days) was recorded for P_8 genotype.

Table 2. Mean squares of pea traits combined of the two seasons.

sov	df	MS								
		PH	FD	Pod L	Pod W	Pod/P	1000sw	Seed/Pod	No Br	Pod Wd
Year (Y)	1	190.04**	359.84**	9.09**	9.90**	8.58**	172.44**	2.99**	0.258	0.0421**
R/Y	4	11.43	4.09	1.32	0.19	1.14	4.24	0.40	1.668	0.005
Genotype (G)	14	3219.23**	581.61**	38.06**	10.06**	803.92**	683.30**	12.50**	14.379**	0.312**
G x Y	14	26.57**	108.20**	7.21**	2.33**	45.45**	26.99**	1.39**	1.253**	0.027**
error	56	8.52	2.22	1.02	0.90	3.21	3.47	0.72	1.049	0.008

Table 3. Mean performance of agro-morphological parameters of pea.

Varieties	PH	FD	Pod L	Pod W	Pod/P	100sw	Seed/Pod	No Br	Pod Wd
P_1	86.74	65.22	7.28	5.78	38.50	53.78	6.68	4.42	1.22
P_2	83.32	58.72	8.51	4.29	39.40	57.41	8.16	4.55	1.15
P_3	57.59	50.20	11.78	5.87	29.22	48.78	9.31	7.11	1.37
P_4	58.69	54.32	7.76	5.97	25.01	79.63	7.73	5.86	0.83
P_5	62.97	55.53	15.50	6.92	55.34	52.44	10.82	5.15	1.37
P_6	37.63	66.83	8.65	4.93	40.75	48.56	7.10	3.66	0.84
P_7	56.22	59.30	10.71	4.52	58.67	35.37	10.27	4.85	1.14
P_8	69.38	39.33	9.34	6.70	37.81	57.03	10.18	5.89	0.84
P_9	74.16	40.17	9.79	7.78	51.54	59.95	10.33	6.37	0.71
P_{10}	56.76	58.17	7.24	4.70	43.78	45.48	7.22	4.99	1.07
P_{11}	119.56	58.05	10.47	4.18	53.60	42.19	9.58	3.84	1.10
P_{12}	82.36	54.01	13.05	6.30	66.00	54.11	10.43	5.22	0.78
P_{13}	91.40	65.20	7.85	3.00	55.91	40.34	4.16	7.96	1.37
P_{14}	104.62	77.16	14.25	5.72	57.13	49.76	10.38	5.65	0.85
P_{15}	111.64	64.61	11.97	7.06	45.54	63.47	9.17	8.69	1.14
LSD 0.05%	1.979	1.184	0.671	0.619	1.206	1.206	0.757	0.756	0.041

The genotype P_5 showed the maximum mean values for pod length (15.5 cm), number of seeds/pod (10.82) and pod width (1.37 cm) while the minimum mean values were recorded for P_{10} (7.24 cm), P_{13} (4.16) and P_9 (0.71 cm), respectively. The maximum mean value of pod weight (7.78 g) was recorded for the genotype P_9 while the minimum value was recorded for P_{13} (3.00)**.** The genotype P_4 revealed the highest mean value of 100 seed weight (79.63g) and the lowest mean number of pods/plant (25.01) while the lowest mean value of 100 seed weight (35.37g) and the highest mean

number of pods/plant (66) were recorded for P_7 and P_{12}, respectively. The mean number of branches/plant ranged from 3.66 (P_6) to 8.69 (P_{15}).

Estimates of Variance Components

Table (4) showed that the genotypic variance (δ^2g) was higher than the environmental one for plant height (δ^2g = 106.42, δ^2e = 2.84), flowering date (δ^2g = 15.78, δ^2e = 0.741), pod length (δ^2g = 1.028, δ^2e = 0.342), pod per plant (δ^2g = 25.282, δ^2e =1.066), 100 seed weight (δ^2g = 21.88, δ^2e = 1.156), seeds per pod (δ^2g = 0.616, δ^2e = 0.371), number of branches per plant (δ^2g = 0.370, δ^2e = 0.239) and pod width (δ^2g = 0.009, δ^2e =0.003).

Phenotypic and Genotypic Coefficients of Variation

The quantitative measurement of individual character provides the basis for an interpretation of different variability parameters. In this study estimation of phenotypic and genotypic coefficient of variation for the nine characters were presented in Table (4). The phenotypic coefficient of variability ranged from 9.877% for 100 seeds weight to 15.979% for pod weight while genotypic coefficient of variability ranged from 6.874% for date to flowering to 13.42% for plant height. According to this interpretation, no high values were recorded for either PCV or GCV.

Estimation of Broad Sense Heritability

The highest heritability was observed for plant height (94.8%) followed by 100 seesds weight (81.2%), number of pods per plant (75.7%), pod width (62.2%) and number of branches per plant (62.2%). Number of seeds per pod (48.6%), flowering date (46.2%), pod length (42.8%) and pod weight (32.4%) shows moderate level of heritability which may be the influence of environment on polygenic nature of these characters (Table 4).

The expected genetic advance expressed as a percentage of the mean (assuming 5% intensity of selection). In this study genetic advance as percentage of mean varied from 0.158 % for pod width to 20.69% for plant height (Table 4), indicating that selecting the top 5% of the base population could result in an advance of 0.158 to 20.69 percent over the respective population mean. The range for genetic advanced mean was from 0.158% (pod width) to 20.69% (plant height). However, plant height had the highest genetic advance (20.69%) as percent of mean in this study, the Remaining traits showed low genetic advance as percentage of mean.

Table 4. Estimation of genetic parameters in fifteen pea genotypes based on nine agro-morphological traits.

	PH	FD	Pod L	Pod W	Pod/P	1000sw	Seed/Pod	No Br	Pod Wd
V.E	2.842	0.741	0.342	0.300	1.066	1.156	0.371	0.239	0.003
V.ExG	3.008	17.662	1.030	0.238	7.041	3.905	0.280	0.112	0.003
V. G	106.42	15.78	1.028	0.258	25.282	21.880	0.616	0.370	0.009
V.P	112.272	34.184	2.400	0.795	33.390	26.941	1.267	0.721	0.015
h^2b	0.948	0.462	0.428	0.324	0.757	0.812	0.486	0.514	0.622
GCV %	13.420	6.874	9.932	9.097	10.802	8.901	8.952	10.883	9.279
PCV %	13.784	10.118	15.173	15.979	12.414	9.877	12.839	15.178	11.761
GA	20.690	5.560	1.367	0.595	9.013	8.684	1.127	0.899	0.158
GG%	26.916	9.622	13.392	10.668	19.364	16.524	12.859	16.076	15.080

Phenotypic Correlation Between agro-morphological traits

Phenotypic correlations are presented in Table (5). Days to flowering was significantly and positively correlated with plant height (r=0.257) and pods/plant (r=0.212), while significantly and negatively correlated with pod weight (r=-0.44), 100 seeds weight (r=-0.297) and seeds/pod (r=-0.317). Pod length had positive and significant phenotypic correlations with pod weight, pods/plant and seeds/pod (r=0.487, 0.414 and 671, p<0.01, respectively)

Pod weight showed positive correlation with 100 seeds weight and seeds/pod (r=0.499, 0.619, p<0.01, respectively) and negative correlation with pod width (r=-0.328, p<0.01).

Pods/plant showed significant and positive correlation with seeds/pod (r = 0.266, p<0.01) and negative correlation with 100 seeds weight (r = -0.477, p<0.01)

The correlation coefficient of pod width was found significant and negative with 100 seeds weight (r=-0.357) and seeds/pod (r=-0.29).

Table 5. Estimates of correlation coefficients among nine agro-morphological traits in pea.

	PH	FD	Pod L	Pod W	Pod/P	100 seed W	Seed/Pod	No Br	Pod Wd
PH	1.00	0.257**	0.188	0.023	0.335**	-0.015	0.038	0.238*	0.101
FD		1.00	0.075	-0.440**	0.212*	-0.297**	-0.317**	-0.018	0.165
PodL			1.00	0.487**	0.414**	-0.064	0.671**	0.060	0.044
Pod W				1.00	-0.091	0.499**	0.619**	0.198	-0.328**
Pod/P					1.00	-0.477**	0.266**	-0.114	-0.066
100 seed W						1.00	0.075	0.199	-0.357**
Seed/Pod							1.00	-0.079	-0.291**
No Br								1.00	0.152
Pod Wd									1.00

***Significant, ** highly significant**

Cluster Analysis of Agro-Morphological Traits

In the present study, the nine agro-morphological traits were adopted based on the Euclidean distances among the 15 genotypes of pea to construct a UPGMA dendrogram as in Fig. 1. The Euclidean distance between all pairs of genotypes ranged from 1.66 between P_8 and P_9 to 6.53 between P_9 and P_{13}. The average of distances among varieties was 4.13.

The dendrogram classified the 15 pea genotypes into five clusters. Cluster I included six pea genotypes, P_1, P_2, P_6, P_7, P_{10} and P_{11} which consisted of 40% of total genotypes. Within this cluster P_1 was closely related to P_2 with the lowest genetic distance. Cluster I included the genotypes which characteristic with a minimum number of branches per plant. Cluster II comprised P_3 and P_5 genotypes, which characterized with the highest mean values of pod width trait

(1.32 cm and 1.37 cm, respectively) compare with the genotypes in the other clusters. Cluster III contained the genotypes, P_{12}, P_{14} and P_5 which showed the highest average for two traits, Pod length and number of seeds per pod. Cluster IV included P_4, P_8 and P_9 genotypes. Within cluster IV, P_8 and P_9 genotype had the lowest average values for day to 50% flowering (39 days and 40 days, respectively).

Cluster V contained P_{13} genotype, characteristic with the lowest averages of pod weight and number of seeds per pod.

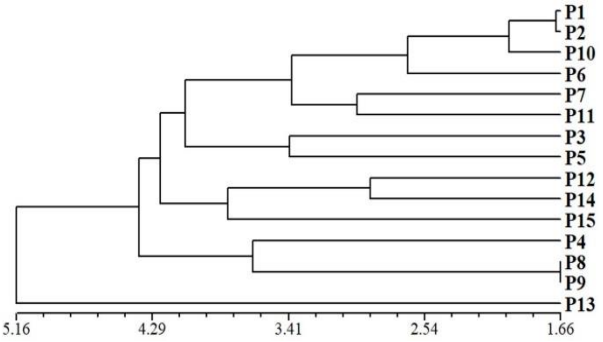

Fig. 1. Dendrogram of 15 pea genotypes based on nine agro-morphological traits using Euclidean distance.

Molecular Diversity

The ten ISSR primer and 10 SRAP primer combinations were used for generating genetic variability among fifteen field pea genotypes (Fig. 2a, b and 3a, b).

ISSR analysis

The ISSR primer amplified a total of 126 amplicons (bands) with a mean of 12.6 amplicons/primer. The number of amplification amplicons obtained with ISSR primers ranged from 9 (HB06) to 17 (UBC814) and which varied in size from 74 bp (UBC812) to 1508 bp (UBC844) (Fig. 2a, b).

Of the 126 bands, 97 were polymorphic with an average of 9.7 polymorphic amplicons /primer. Polymorphism ranged from 44.44% (HB06) to 88.24% (UBC814) with an average of 76.97%.

Table 6 showed that the PIC value of the ten ISSR primers varied from 0.12 (HB11) to 0.33 (UBC818) with an average of 0.22. The MI varied from 0.59 (HB06) to 4.53 (UBC814), with the mean of 2.13. The highest RP value was recorded for HB10 primer (4.93) while the lowest value (3.4) was obtained by HB09 and UBC818 primers, with overall primers average 4.07

The genetic similarities (GS) of 15 pea genotypes based on the ISSR markers using Dice similarity coefficient ranged from 0.657 (P_2 and P_8) to 0.87 (P_5 and P_6) with an average of 0.761. The dendrogram (Fig. 2b) classified the 15 pea genotypes into two main clusters at the 0.75 The first sub-cluster Ia comprised P_1, P_2, P_3, P_4, P_5 and P_6. Sub-cluster Ib contained P_7 with genetic similarity 0.781 with genotypes in the same cluster. Cluster II consisted of 8 genotypes divided into three sub-clusters. The genotypes P_8 and P_9 formed Sub-cluster IIa. Sub-cluster IIb consisted of P_{10}, P_{11}, P_{12}, P_{14} and P_{15} genotypes. The genotype P_{13} formed sub-clusters IIc which separated from the other genotypes in cluster II with genetic similarity 0.746.

Mantel's test based on matrices generated from ISSR marker and standardized agro-morphological data showed a significant positive correlation ($r=0.267$: $p \leq 0.001$).

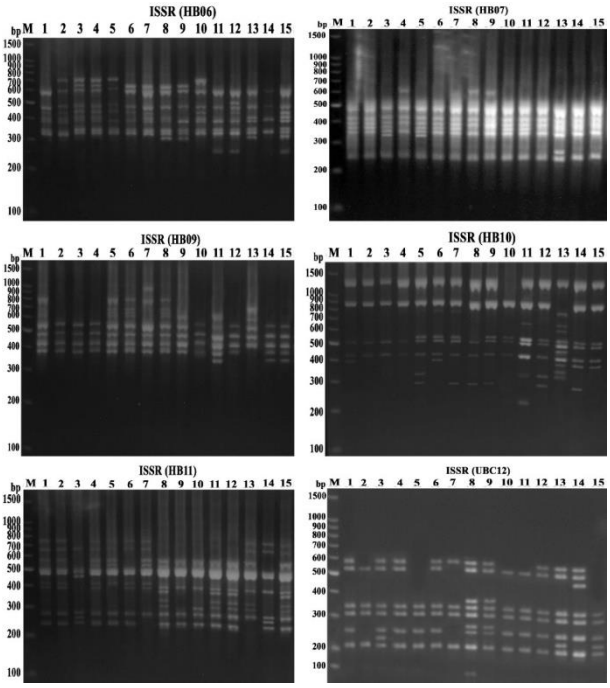

Fig. 2a. Electrophoresis patterns of The six ISSR markers of the15 pea genotypes.

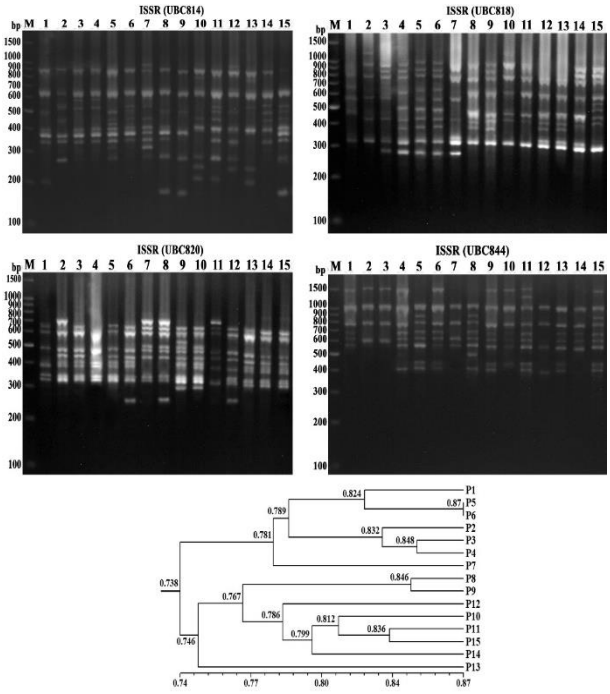

Fig. 2b. Electrophoresis patterns of The four ISSR markers of the15 pea genotypes. The dendrogram of 15 pea genotypes developed from 10 ISSR primers

SRAP analysis

Ten SRAP primer combinations that produced clear polymorphic amplicons were screened across the fifteen pea genotypes for genetic diversity analysis. A total of 147 amplicons were amplified across the 15 tested genotypes. The number of amplification bands obtained with the SRAP primer combinations ranged from 9 (SRAP-1) to 19 (SRAP-6) with an average of 14.7 amplicons/primer. The amplicon size ranged from 159 bp (SRAP-6) to 1721 bp (SRAP-3) (Fig. 3a, b). Of 147 amplified amplicons, 116 amplicons were

polymorphic, the number of polymorphic amplicons ranged from 7 (SRAP-8) to 15 (SRAP-2) with an average of 11.6 polymorphic amplicons per primer. The highest percentage of polymorphic amplicons (93.33%) amplified with SRAP-4 primer while the lowest percentage of polymorphic amplicons was obtained by primer SRAP-8 (63.64%) with an average of 78.91 % (Table 6).

In this study, the highest PIC value (0.31) obtained by the combination SRAP-2 and the lowest value (0.15) obtained by combination SRAP-1, with the average value being 0.23. The overall mean MI was estimated as 2.76, with the highest value observed in SRAP primer SRAP-2 (4.61) and lowest in SRAP primer SRAP-1 (0.75). The RP of the SRAP primer combinations ranged from 3.63 for SRAP primers SRAP-1 to 5.87 for SRAP primers SRAP-9 and SRAP-10. The average RP value was determined as 4.92 (Table 6).

The genetic similarities (GS) of 15 genotypes ranged from 0.652 (P_2 and P_8) to 0.865 (P_8 and P_9) with an average value of 0.751. The dendrogram of 15 tested genotypes is shown in Fig. 3b. The 15 genotypes were grouped in three main clusters at the 0.734 similarity coefficient level. Cluster I contained P_1, P_2, P_3 and P_4. Cluster II included the highest number of pea genotypes (8 genotypes) and divided into three sub-clusters. The first sub-cluster IIa comprised P_4, P_5, P_6 and P_7. Sub-cluster IIb contained P_8, P_9 and P_{12}. The genotypes P_{10} and P_{11} formed sub-cluster IIc. Within cluster II, P_8 and P_9 genotypes were closely related together with 0.865 similarity coefficient. Cluster III consisted of P_{13}, P_{14} and P_{15}.

Mantel's test showed a positive and significant correlation ($r = 0.421$: $p \leq 0.001$) between SRAP marker and standardized agro-morphological data.

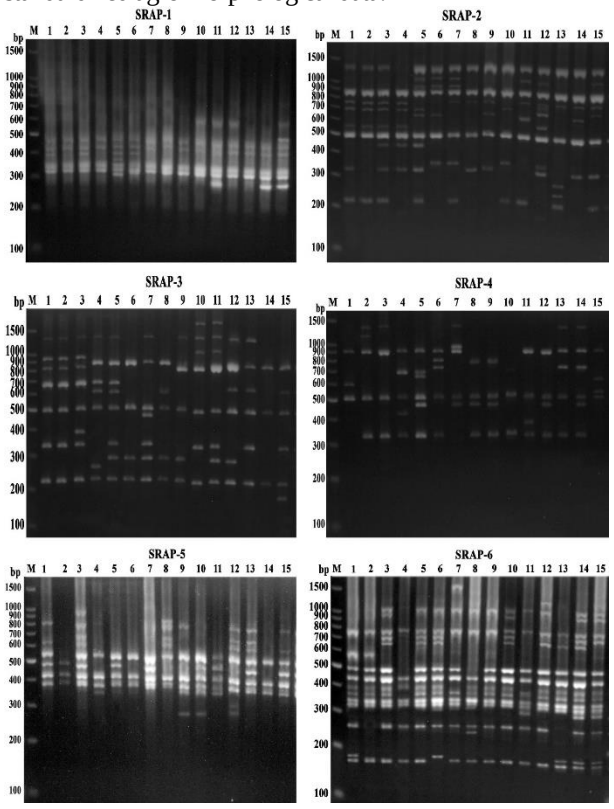

Fig. 3a. Electrophoresis patterns of The six SRAP markers of the15 pea genotypes.

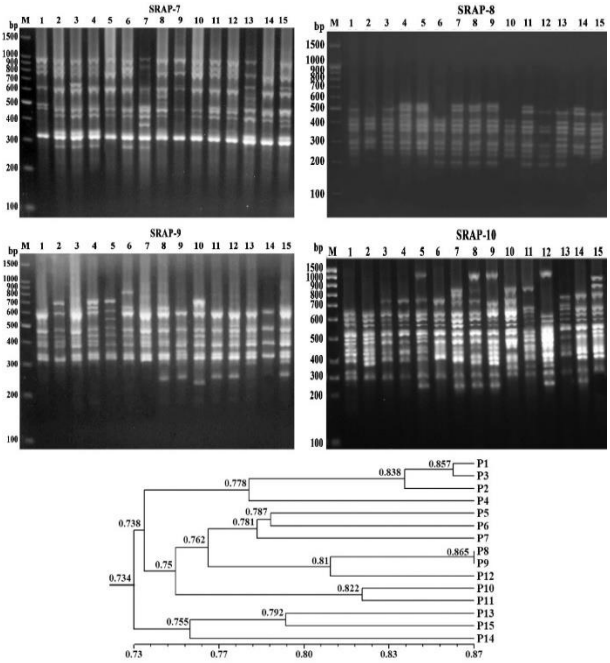

Fig. 3b. Electrophoresis patterns of The four SRAP markers of the15 pea genotypes. The dendrogram of 15 pea genotypes developed from 10 SRAP primers

Combined ISSR and SRAP data analysis

In general, ISSR and SRAP primers or primer combinations generated a total of 273 amplicons with an average of 13.65 amplicons/primer. Of the 273 amplicons, 213 (78.02%) were polymorphic, with an average of 10.65 polymorphic amplicons/primer.

Table (6) showed that the average values of PIC, MI and RP were 0.23, 2.45 and 4.49 for all primers, respectively. The two genotypes, P_5 and P_{15} possessed the highest number of generated bands (159 bands) from both ISSR and SRAP markers followed by P_8 (151 bands) and P_{13} (149 bands) while P_2 have the lowest number (124 bands).

Genetic similarity of combined ISSR and SRAP data ranged from 0.655 (P2 and P8) to 0.855 (P8 and P9) with an average of 0.755.

The dendrogram of combined ISSR and SRAP markers across the 15 pea genotypes is represented in Fig. 4.

The dendrogram revealed that the genotype P_{13} was separated in a single branch from the other genotypes within 72.7% branched-off genetic similarity while the other genotypes were grouped into two major clusters. Cluster I comprised of 7 genotypes, representing 46.67% of total genotypes. Within cluster I, the genotypes are further divided into two sub-clusters. Sub-cluster Ia consisted of P_1, P_2, P_3 and P_4 with a mean genetic similarity 79.9%. Sub-cluster Ib comprised P_5, P_6 and P_7 with a mean genetic similarity 78.8%. Cluster II contained the remaining pea genotypes which representing also 46.67% of total genotypes. The genotypes within cluster II are feature divided into two sub-clusters. Sub-cluster IIa consisted of P_{10}, P_{11}, P_{14} and P_{15}. Sub-cluster IIb contained three genotypes P_8, P_9 and P_{12}, within this sub-cluster P_8 and P_9 genotypes were closely related to each other, with a 0.855 genetic similarity.

Mantel's test showed significant positive correlation between the matrices generated from ISSR and SRAP data was ($r = 0.389$: $p \leq 0.001$), indicating the existence of a quite

compatibility between ISSR and SRAP marker systems. Also, the Mantel Z test statistics showed significant positive correlation ($r = 0.411$: $p \leq 0.001$) between the matrices of combined molecular markers and agro-morphological data

ISSR and SRAP marker systems were efficient in characterizing all genotypes by unique positive and/or negative bands (Table 7). The two molecular markers showed 55 genotypes-specific markers that represent 25.82% from the polymorphic band detected and 20.15% from the total band numbers (Table 7).

The two genotypes P_{12} and P_{13} possessed the highest number of positive and/or negative genotype-specific markers (7 for each) followed by P_4 (6 markers) and P_7 (5 markers) while the genotype P_6 recorded the lowest number (1 marker).

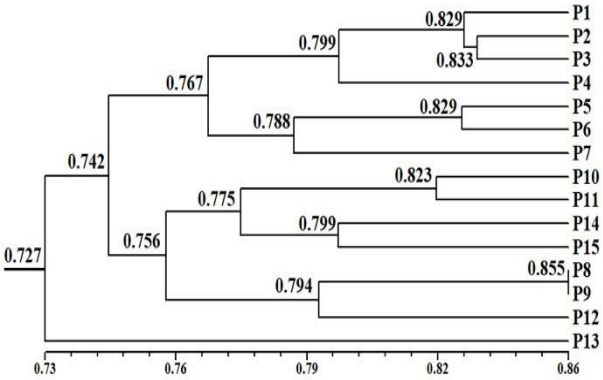

Fig. 4. The dendrogram of 15 pea genotypes developed from the combined molecular markers data.

Table 6. Molecular parameters of ISSR primers and SRAP primer combinations used for 15 pea (*Pisum sativum* L.) genotypes analysis.

Markers	TB	PB	PPB	PIC	MI	RP
HB06	9	4	44.44	0.15	0.59	3.50
HB07	12	8	66.67	0.24	1.94	3.87
HB09	10	7	70.00	0.22	1.56	3.40
HB10	14	12	85.71	0.22	2.62	4.93
HB11	15	12	80.00	0.12	0.28	4.43
UBC820	13	10	76.92	0.21	2.12	4.70
UBC812	11	8	72.73	0.18	1.47	4.23
UBC814	17	15	88.24	0.30	4.53	4.83
UBC818	14	12	85.71	0.33	3.95	3.40
UBC844	11	9	81.82	0.25	2.27	3.43
SUM	126	97				
AVR	12.6	9.7	76.97	0.22	2.13	4.07
Markers	TB	PB	PPB	PIC	MI	RP
SRAP -1	9	5	55.56	0.15	0.75	3.63
SRAP -2	17	15	88.24	0.31	4.61	4.50
SRAP -3	16	14	87.50	0.28	3.90	4.73
SRAP -4	15	14	93.33	0.23	3.19	5.30
SRAP -5	12	10	83.33	0.25	2.55	3.73
SRAP -6	19	14	73.68	0.20	2.83	6.83
SRAP -7	14	12	85.71	0.24	2.83	4.80
SRAP -8	11	7	63.64	0.19	1.35	3.90
SRAP -9	16	12	75.00	0.20	2.35	5.87
SRAP -10	18	13	72.22	0.25	3.26	5.87
SUM	147	116				
AVR	14.7	11.6	78.91	0.23	2.76	4.92
SUM	273	213				
AVR	13.65	10.65	78.02	0.23	2.45	4.49

Table 7. Pea genotypes identified positive and/or negative unique bands using ISSR and SRAP markers

Varieties	Unique Bands	
	Positive	Negative
P_1	SRAP-4_{604bp}	UBC818_{416bp}
P_2	SRAP-4_{1161bp}, UBC$818_{1119\ bp}$	SRAP-5_{527bp}
P_3	HB11_{448bp}, SRAP-3_{390bp}, SRAP-5_{958bp}	--------
P_4	SRAP-3_{261bp}, SRAP-4_{428bp}, SRAP-8_{453bp}	SRAP-2_{1251bp}, SRAP$6_{465,\ 350bp}$
P_5	SRAP-$1_{366,\ 306bp}$, UBC814_{418bp}, SRAP-9_{553bp}	--------
P_6	SRAP-9_{827bp}	--------
P_7	HB09_{954bp}, SRAP-3_{482bp}, SRAP-4_{990bp}, SRAP-6 $_{1586bp}$	SRAP-7_{741bp}
P_8	UBC812_{74bp}, SRAP-5_{840bp}, SRAP-6_{229bp}	--------
P_9	UBC820_{522bp}, SRAP-9_{356bp}	--------
P_{10}	SRAP-9_{235bp}	HB10_{1280bp}
P_{11}	HB10_{226bp}, SRAP-4_{387bp}, UBC844_{1275bp}	UBC820_{334bp}
P_{12}	HB07_{497bp}, SRAP5_{298bp}, SRAP-6_{1128bp}, SRAP-9_{500bp}	SRAP-$8_{414\ bp}$, UBC844_{621bp}, SRAP-$10_{647\ bp}$
P_{13}	HB06_{265bp}, HB$10_{585,\ 339}$, SRAP-$2_{250bp,\ 222bp}$	HB09_{380bp}, HB10_{888bp}
P_{14}	UBC812_{357bp}	SRAP-$7_{932\ bp}$
P_{15}	SRAP-3_{164bp}, SRAP-4_{555bp}, SRAP-10_{925bp}	UBC814_{872bp}

Discussion

Understanding the nature and the extent of diversity in genotypes is of prime importance for a breeder since it provides the establishment for selection (Tyagi and Singh, 1998).

In this investigation, 15 diverse genotypes of pea obtained from different sources were studied to assess their

genetic potential for agro-morphological traits. The combined analysis of variance due to genotype was highly significant for all studied traits, thereby suggesting the presence of considerable amount of variability among tested genotypes and this variability can be further utilized in the pea improvement program by selecting superior and desired genotypes (Gupta *et al.,* 2020). These results in concordance with the works of Dagla *et al.* (2013), Kumar *et al.* (2013), Gixhari *et al.* (2014), Ouafi *et al.* (2016), Kumar *et al.* (2019), and Gupta *et al.* (2020) who analyzed genetic diversity among different genotypes of pea using the same agro-morphological traits and found significant differences. Also, the differences were significant for the factor year and the interaction "genotype x year". The significance of years and genotype/year interaction variances indicated that the studied genotypes interacted differently with the tested seasons. These results in agreement with those obtained earlier by Espósito *et al.* (2009), Dagla *et al.* (2013), Kumar *et al.* (2013), Chikezie *et al.* (2016) and Ouafi *et al.* (2016).

The estimates of genotypic variation (δ^2g), phenotypic variation (δ^2p), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2b) and genetic advance (GA) for studied traits have been presented in Table 4. The magnitude of genotypic variances was higher than their corresponding environmental variances for all the traits, except for pod weight that was very negligible. This indicates that a negligible role was played by the environmental factors in the inheritance of these traits in pea genotypes. The high genotypic variance for most of these traits was also reported by other researchers (Gixhari *et al.*, 2014; Ouafi *et al.*, 2016; Kumar *et al.*, 2019 and Gupta *et al.*, 2020).

While, the genotype/year interaction variance was also important in the expression of plant height, days to flowering, pod length, number of pods per plant and 100 seeds weight which they displayed δ^2gy values higher than δ^2e (Table 4). These results in outline with (Gixhari *et al.*, 2014 and Ouafi *et al.*, 2016).

Among the variability parameters studied are genotypic (GCV) and phenotypic (PCV) coefficient of variations, the first parameter indicated the magnitude of variations that exclusively due to the gene action, whereas the latter indicated the total variations generated and is attributed to environmental component along with the genotypic variations. The study of PCV and GCV is not only useful for comparing the relative amount of phenotypic and genotypic variations among different traits but also very useful to estimate the scope for improvement by selection. Our study revealed that the PCV was higher than the GCV for all studied traits. This was also the case for all the traits observed in previous studies (Gudadinni *et al.*, 2017; Kumar *et al.*, 2019; Gupta *et al.*, 2020), which reported that the environmental effect on any trait is indicated by the magnitude of the differences between the genotypic and phenotypic coefficients of variation; large differences reflect a large environmental effect, whereas small differences reveal a high genetic influence.

In this study, the low differences between the PCV and GCV for some traits, such as plant height, pods/plant, 100 seeds weight and pod width indicated less environmental influences on the phenotypic expression of these traits. In other words, it seems that genetic factors were predominantly responsible for expression of these traits and suggests that selection based on phenotypic performance of these traits would be effective for future crossing programs. The other traits, which showed a higher difference between PCV and GCV, indicated that the environmental effect on the expression of those traits is higher and that selection based on phenotypic performance of these traits is not effective for further yield improvement. The finding of Gixhari *et al.* (2014), Ouafi *et al.* (2016), Yumkhaibam *et al.* (2019) and Gupta *et al.* (2020) were similar to the present findings.

In this study, the high heritability estimates (62.2 to 94.8%) for plant height, 100seeds weight, number of pods per plant and pod width indicated that there is great genetic variation present in these traits to insure selection for best accessions. These traits can therefore be given special attention for selections aimed at pea improvement.

The moderate level of heritability (42.8 to 51.4%) for number of branches per plant, number of seeds per pod, flowering date and pod length reflects that may be due to the influence of environment on polygenic nature of these traits. When heritability in broad sense is low the characters are highly influenced by the environment, therefore, genetic improvement through selection will be difficult. Similar finding was observed by Gixhari *et al.* (2014) and Kaur *et al.* (2018).

The genetic advance is a useful indicator of the progress, it helps to predict the extent of improvement that can be achieved for improving the different characters. Heritability in conjunction with expected genetic gain is more useful than the heritability value alone in predicting the resultant effect for selecting the best genotypes (Johnson *et al.*, 1955). Maximum genetic gain (assuming 5% intensity of selection) along with high heritability was observed for plant height (GG = 26.92%, h^2 = 94.8), reflecting the preponderance of additive gene action in determining this trait. This also provides the evidence that larger proportion of phenotypic variance has been attributed to genotypic variance, and reliable selection could be made for these traits on the basis of phenotypic expression. These results find support from the earlier studies by Dagla *et al.* (2013), Georgieva *et al.* (2016), Gudadinni *et al.* (2017), Kumar *et al.* (2019) and Yumkhaibam *et al.*, (2019).

The moderate genetic gain associated with high amount of heritability for number of pods per plant (GG = 19.36%, h^2 = 75.7%), 100 seeds weight (GG = 16.52%, h^2 = 81.2%) and pod width (GG = 15.08%, h^2 = 62.2%), indicating that non-additive gene action governing these traits, and these traits could be improved through the use of hybridization and hybrid vigor. Meanwhile, the most critical point is that high heritability causes for these traits might be due to prevailing of favorable environmental conditions during the seasons rather than genetic cause. The findings were in agreement to the findings of Singh *et al.* (2011), Dagla *et al.* (2013) and Kumar *et al.* (2013).

The moderate genetic gain associated with moderate heritability for pod length (GG = 13.39%, h^2 = 42.8%), seeds per pod (GG = 12.86%, h^2 = 48.6%), pod weight (GG = 10.67%, h^2 = 32.4%) and number of branches per plant (GG = 16.08%, h^2 = 51.14%) indicating that there exists a scope to improve this character to a considerable extent by adopting suitable breeding procedures.

Low genetic gain with moderate amount of heritability were observed for flowering date (GG = 9.62%, $h^2 = 46.2$), indicate that the influence of error variance on such trait is high and suggested a low scope in the improvement of this trait. As this trait also exhibited low genotypic and phenotypic coefficient of variations, therefore, improvement by direct selection may not be possible, but through indirect selection of other correlated traits may be feasible. These findings collaborated with the earlier findings of Dagla *et al.* (2013) and Kumar *et al.* (2019).

The Euclidean distance was relatively wide reflecting the genetic variation of the loci controlling these agro-morphological traits. Therefore, the results of the genetic distance have shown that there is a room for the genetic improvement of these pea genotypes and the information generated can be utilized to make broad crosses and release new varieties. These findings collaborated with the earlier findings of Yirga *et al.* (2013), Gixhari *et al.* (2014), Georgieva *et al.* (2016), Arif *et al.* (2018), Hanci (2019), Kumar *et al.* (2019), Mohamed *et al.* (2019) and Yumkhaibam *et al.* (2019).

In our study, the molecular diversity of 15 pea genotypes was analyzed using ISSR and SRAP markers. The use of different molecular marker systems give chance to coverage a great parts of the genome and provides more information than the use individual markers (Elmeer *et al*., 2017). In this study, ten ISSR primers generated a total of 126 reproducible bands, and the ten SRAP primer combinations amplified 147 bands. The percent polymorphic band obtained using SRAP (78.91%) were a bit higher than obtained by the ISSR marker (76.98%). This may be return to that these markers targeting different sequences of the genome. Also, the mean number of total bands and polymorphic bands detected by each SRAP primer combinations was 14.7 and 11.6, respectively, which are a bit higher compared to the ISSR primers (12.6 and 9.7, respectively). Similar finding were found by Esposito *et al.* (2007), Pakseresht *et al.* (2013), Kole *et al.* (2015), Guindon *et al.* (2016). In general, the combined analysis of two markers revealed high polymorphism (78.02%). Sivaprakash *et al*. (2004) suggested that the percentage of polymorphisms generated by the molecular marker systems possessed ability to resolve genetic diversity between different genotypes

The high level of polymorphism rate indicates the genetic divergence among the studied genotypes and makes these marker systems ideal for study genetic diversity among pea genotypes.

The PIC is one of the most important genetic marker parameters in the strategy of breeding programs, it give more information of polymorphism among and/or within germplasm (Chesnokov and Artemyeva, 2015). In this study, the average value of PIC obtained by SRAP marker (0.23) was a bit higher than obtained by ISSR marker (0.22) however; both ISSR and SRAP markers were equally efficient in detection of polymorphisms among pea genotypes. Similar results are obtained by Baranger *et al.* (2004), Esposito *et al.* (2007), Pakseresht *et al.* (2013), Kole *et al.* (2015) and Guindon *et al.* (2016).

Results showed slight difference between ISSR and SRAP markers in the average of MI values of ISSR and SRAP mmarkers used in this study difference between the marker systems used in the present study 2.76 (SRAP) and 2.13(ISSR), which is in agreement with the results of (Baranger *et al.*, 2004; Esposito *et al.*, 2007; Kapila *et al.*, 2012; Pakseresht *et al.*, 2013; Kole *et al.*, 2015 and Guindon *et al.*, 2016). The average values of RP index showed a small difference between SRAP (4.92) and ISSR (4.07) markers. Similar finding were obtained by (Baranger *et al.*, 2004; Esposito *et al.*, 2007; Pakseresht *et al.*, 2013; Kole *et al.*, 2015 and Guindon *et al.*, 2016).

The dendrogram based on each of ISSR and SRAP markers indevidual, as well as their combined data for the 15 genotypes of pea were relatively similar. Where the dendrogram constructed based on combined matricses of ISSR and SRAP markers was more similar withthe dendrogram obtained from SRAP matrix than the dendrogram obtained from ISSR matrix.

The highest genetic similarity values between these genotypes were 0.848 (ISSR), 0.865 (SRAP) and 0.86 (combined ISSR and SRAP). The highest similarity were between the P_8 and P_9 genotypes [0.865 for SRAP and 0.860 for the combined data]; While the ISSR data revealed that the highest similarity was recorded between P_5 and P_6 genotypes. The high genetic similarity between these genotypes may be due to the existence resemblance in the amplified region.

Mantel test showed significant correlation between molecular markers and agro-morphological data ($r=0.411$: $p\leq 0.001$). These finding in outline with several studies by Kwon *et al.* (2012), Kole *et al.* (2015), Kour *et al.* (2016), who's revealed the efficiencies of these markers in screening of genetic diversity in pea germplasm.

CONCLUSION

Our results indicated the existence of considerable genetic variations at agro-morphological and molecular levels. In addition, the two molecular markers were effective in estimated of genetic diversity among pea genotypes. Also, these markers were able to distinguish the pea genotypes with high sufficiency and accuracy. So, we suggest use of these genotypes of pea in breeding programs to obtain hybrid vigor and release new varieties.

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تقييم العلاقات الجينية والتنوع الجيني بين الطرز الوراثية للبازلاء (*Pisum sativum* L) باستخدام الواسمات المورفولوجية الزراعية والجزيئية

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تم تقييم خمسة عشر طرازا وراثيا من البازلاء (*Pisum sativum* L) لأدائها المورفولوجي الزراعي والتنوع الجزيئي خلال موسمي 2017-2018 و 2018-2019. أظهر تحليل التباين المشترك وجود فروق معنوية بين الطرز الوراثية للبازلاء المختبرة لجميع الصفات المورفولوجية الزراعية. كان معامل الاختلاف المظهري (PCV) أكبر نسبيًا من معامل الاختلاف الوراثي (GCV) لجميع الصفات ؛ ومع ذلك ، كانت قيم GCV قريبة من قيم PCV لبعض الصفات ، مثل ارتفاع النبات ، عدد القرون لكل نبات ، وزن 100 بذرة وعرض القرون. تم الحصول قيم عالية لمعامل التوريث بالمعنى العريض مع قيم عالية للتقدم الوراثي بالنسبة لارتفاع النبات ، مما يعكس أهمية فعل الجينات المضيفة في وراثة هذه الصفة. كما أظهرت النتائج وجود قيم عالية ومتوسطة لمعامل التوريث مع قيم متوسطه للتقدم الوراثي بالنسبة لعدد القرون لكل نبات ، ووزن 100 بذرة ، وطول القرون ، والبذور لكل قرنة ، ووزن القرون ، وعدد الأفرع لكل نبات. المسافة الإقليدية بين جميع الطرز الوراثية المعتمدة على الصفات المورفولوجية الزراعية كانت واسعة نسبيًا. تم استخدام نظامين للواسمات الجزيئية ، ISSR و SRAP لدراسة التنوع الوراثي بين الطرز الوراثية للبازلاء. أظهرت النتائج أن واسمات الـ SRAP كانت أكثر كفاءة من واسمات الـ ISSR فيما يتعلق بتعدد الأشكال ، ومتوسط عدد الحزم متعددة الأشكال لكل بادئ (PB) ، وقدرة التمييز (RP) ، ودليل الواسم (MI) ومحتوى معلومات تعدد الأشكال (PIC). واسمات الـ ISSR و الـ SRAP أظهرت حزم DNA فريدة ومميزه قادرة على تمييز الطرز الجينية الأكثر تنوعًا. أظهر اختبار Mantel وجود ارتباط معنوي موجب بين كل نت الـ ISSR و الـSRAP ($r = 0.389: p \leq 0.001$). أيضًا ، ارتباط معنوي ($r = 0.411: p \leq 0.001$) بين الواسمات الجزيئية والصفات المورفولوجية الزراعية.