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Genetic linkages of anthocyanin pigmentation with seed phenotype and root architecture with yield contributing traits, detected in edible pea (*Pisum sativum* L.)

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Abstract

Genetic diversity and trait correlation in pea germplasm is useful for plant breeding programs, which helps to select genetic material. Based on anthocyanin pigmentation (presence/absence), 24 pea competent lines were segregated into two major lineages. These lineages were sub-divided into 4 clusters based on seed shape and seed coat coloration. Similarly, the strong genetic linkage was detected in root morphological characters with yield contributing traits. The trait association was confirmed through cluster analysis, scattered plots, Kruskal Wallis test and Person's correlation. The results found that root length is positive and significant correlated with plant height and 100 seed weight. Likewise, the number of lateral roots showed genetic linkage with number of pod plant^{-1}, pod weight plant^{-1}, grain yield plant^{-1} and total biomass. Similarly, root biomass showed genetic linkage with 100 seed weight and plant height. While, root phenotype (15 and 30 days old plant) not showed significant correlation with the number of branch plant^{-1} and number of seed pod^{-1}.

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Introduction

Pea is an essential food legume, growing in temperate and sub-tropical regions. Pea is a rich source of dietary protein and consumed either fresh or dry seed (Nemecek *et al.*, 2008). It is growing in rotation with cereal crops and plays a significant role in soil fertility by fixing atmospheric nitrogen through roots by *Rhizobacteria* (Negi, 2006). While facing the climatic challenges, the need for biotic and abiotic stress resistance pea lines development is the cry of the day, which rely on exploitation of genetic resources that can be achieved either from wild or cultivated gene-pool of *Pisum sativum* germplasm (Burstin *et al* 2015).

Genetic diversity is a prerequisite for any breeding program, which provides opportunity to a plant breeder for selection of high yielding genotypes and for stabilizing production in the face of disease epidemic and fluctuation environmental condition (Saleem *et al.*, 2005; Ali *et al.*, 2007). The evaluation of genetic diversity and its relationship within genotype collections are also useful for facilitating competent germplasm collection, management and utilization (Nisar *et al.*, 2008). The study of genetic diversity and genetic linkage based on quantitative and qualitative traits is a common practice in agricultural crops, viz it was successfully used in Lentils (Bakhsh *et al.*, 20013); chickpea (Gaur *et al.*, 2012); pea (Sultana *et al.*, 2005).

Anthocyanins are chemical compounds of plant origin well known as pigments responsible for blue, purple, red, or orange coloration of plant tissues and organs (Havrlentova *et al.*, 2014). Anthocyanins are generally specified as bioactive and nutritional compounds, responsible for antioxidant and UV/photoprotective functions (Ryan *et al.*, 2001), playing also role in plant protection against stress and helpful in reproduction (Kong *et al.*, 2003). Wrinkled seeds possess high concentration of sucrose, fructose, and glucose levels, and this appears to result in a higher water content in immature seeds due to increased osmotic pressure and hence increase water uptake. While round seeds possess low concentration of sucrose, fructose, and glucose levels and hence decrease water uptake (Reid and Ross, 2011). In addition, the wrinkled seeds contain a higher percentage of lipids as compared to the round seeds (Coxon and Davies 1982). Understanding of the genetic variability and genetic correlation among different traits would help to incorporate desirable traits in pea lines. Therefore the present investigation was designed to study the genetic linkage among anthocyanin pigmentation with seed phenotype in pea lines for selection of farmers and consumer preferred types for cultivation.

Plant growth and yield is correlated with water and nutrient availability. Plants with longer and stronger root system are more efficiently in search of water and inorganic nutrients. High root biomass is beneficial for strong shoot growth, which is essential for high yield production (Qu *et al.*, 2003; Jin *et al.*, 2010). Important morphological traits of the root system involve in absorb water efficiently are the root length, root thickness, the number of lateral root emergence and number of root hairs. The strong root system directly affects the grain yield and total biomass of the pea plant. However, the relationship of root phenotype with yield contribute traits are limitedly analyzed in major crops, particularly in *Pisum* species. Previous pea selection and its ideo-type creation were focused mainly on the aerial parts of the plant (Androsiuk, 2012). Hence forward, in this paper it was further proposed to find the genetic diversity in newly established pea lines and to search genetic linkage of root phenotype with yield contributing traits to derive breeding strategies to develop high yielding candidate line.

Material and method

Plant materials

The present study was conducted on 24 pea lines (PLs); out of which 20 lines (PL-1 to PL-20) were developed from two isogenic parents the "Fallon^{er} (PL-23)" and "11760-3ER (PL-24)" reported by Nisar and Ghafoor (2009). In the remaining four lines: Fallon^{er} (PL-23) and 11760-3ER (PL-24) assessed as parental lines, while the two lines, Climex (PL-22) and Locally-Adopted-Cultivar (PL-21) were used as control.

Experiment and data recording

The experiments were conducted during 2013 and 2014 at the university of Malakand, Khyber Pakhtunkhwa, Pakistan located at Coordinates N 340 40" 318' and E720 03" 753' with 726m altitude. The experiments were carried out in complete randomized block design with three replicates as suggested by Hanaa and Ali (2011).

Qualitative traits

A total of eight qualitative traits was recorded on ten randomly selected plants per pea lines in a row. Qualitative traits observed during the study were: anthocyanin pigmentation (AP) on plant node, flower color, AP on leaf and stem, pod color, plant height, pod shape and seed phenotype (seed shape and seed color). The qualitative morphological traits were coded numerically for biometrical analysis (Table 1).

Root architecture

Pea lines were grown in a small pots to evaluate the level of genetic diversity in root architecture, after 15 and 30 days of seeds germination. For biometrical analysis at different growth stages, the root length (cm), number of lateral roots emergence (by number) and total root biomass (g) were scored on ten randomly selected plants/line.

Yield contributed traits (YCT)

In field experiments eight yield contributed traits: number of branches plant^{-1}, number of pods plant^{-1}, pod weight plant^{-1}(g), number of seeds pod^{-1}, grain yield plant^{-1} (g), 100 seed weight (g), plant height (cm) and plant biomass (g) were scored to estimate the level of genetic diversity and find genetic linkage with root architecture.

Biometrical analysis

To find genetic linkages among "anthocyanin pigmentation with seed phenotype (seed shape and seed color)" and "root architecture with YCT", the Statistical software PC-ORD (McCune and Grace, 2005) were used. The level of genetic diversity and traits association were estimated through Kruskal Wallis test (Kruskal and Wallis, 1952) and Person’s correlation respectively.

Result

Genetic linkage of AP with seed phenotype

Cluster analysis

The Linkage analysis based on Euclidean distances between eight qualitative traits was determined by One Way dendrogram produced by the PC-ORD software.

Table 1. Morphological traits along with their codes scored in 24 pea lines.

S. No.	Traits	Traits characteristics	Code
1	Node colour	White	0
		Dark pink	1
2	Flower colour	White	0
		Pink	1
3	Anthocyanin pigment	Absent	0
		Present	1
4	Pod colour	Green	0
		Brown	1
5	Plant height	Dwarf	0
		Tall	1
6	Pod shape	Straight	0
		Curve	1
7	Seed shape	Round	0
		Wrinkled	2
8	Seed colour	Creamy	0
		Brown	1
		Brownish green	2

Table 2. Genetic linkage of root architecture with plant height extracted from clusture analysis based on Euclidean distances

Root architecture		Genetic linkage of Tall pea lines (58% in L-1)	Genetic linkage of Dwarf pea lines (42% in L2)
Root length	Long root (%)	79 (C-I)	40 (C-II)
	Short root (%)	21(C-III)	60 (C-IV)
Number of lateral roots	High lateral root (%)	57 (C-I & C-II mixed)	57 (C-I & C-II mixed)
	Low lateral root (%)	43 (C-III & C-IV mixed)	43 (C-III & C-IV mixed)
Root biomass	High root biomass (%)	71 (C-I & C-IV mixed)	36(C-III & C-IV mixed)
	Low root biomass (%)	29 (C-III & C-IV mixed)	64 (C-III & C-IV mixed)

The dendrogram distributed 24 lines into 2 lineages (Fig. 1), which was further divided into four clusters. The percentage similarity indexes between pea lines were 60 to 90 %. Lineage 1 (L-1) consists of 14 pea lines with anthocyanin pigmentation observed in different part of the plant body. On the other hand, lineage 2 (L-2) was comprised of 10 pea lines with no anthocyanin pigmentation. At 40% linkage distance, the L-1 was further divided into two clusters; of which cluster-I consists of five lines (1, 5, 11, 15 and 17), which were wrinkled seeded with brownish seed coat. While cluster-II consists of nine lines (2, 7, 8, 9, 16, 18, 19, 20 and 24), which were wrinkled seeded with brown seed coat. Furthermore, L-2 at genetic distance 30% was subdivided into two clusters, Out of which, C-III was comprised of four lines (3, 4, 14, 23), which were round seeded with creamy seed coat. While C-IV of L-2 grouped six pea lines (6, 12, 10, 13, 21 and 22) with wrinkled seeded and creamy seed coat. The genetic association of anthocyanin pigmentation with seed phenotype was reconfirmed through Scatter Plot (Fig. 2). The plot also identified the same genetic association among 24 pea lines.

Table 3. Result of the descriptive statistics and Kruskal Wallis test performed on root architecture and YCT, significant difference were shown in bold.

Traits	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Chi Square	*P*. value
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE		
RL1	94.3±3.90	61±5.57	90±2.12	68.50±3.90	15.03	0.002
RL 2	99.3±3.90	66±5.57	95.75±2.95	67.50±2.09	15.82	0.001
LR1	12.8±0.83	8±1.15	13.50±0.65	9.00±0.89	11.80	0.008
LR2	16.6±1.00	11.33±1.86	18.50±0.87	12.83±1.08	9.83	0.020
RM1	0.77±0.09	0.46±0.16	0.53±0.05	0.62±0.08	4.31	0.230
RM2	1.43±0.07	0.86±0.09	1.23±0.05	0.99±0.05	16.70	0.001
B/P	6.07±0.57	6.45±0.31	5.51±1.51	5.43±0.77	1.69	0.638
P/P	48.62±5.02	46.17±5.78	54.70±5.86	45.79±3.99	1.61	0.657
PW/P	71.27±9.20	72.06±11.81	80.11±9.38	72.19±2.88	0.76	0.858
S/P	6.07±0.26	5.68±0.33	5.67±0.26	5.96±0.22	2.59	0.459
100SW	30.61±1.78	28.95±5.71	27.53±1.00	24.80±2.31	4.31	0.230
GY/P	53.72±7.60	54.26±9.76	57.65±8.23	52.25±4.63	0.78	0.854
PB	152.38±23.93	134.57±31.10	163.50±22.11	142.37±20.92	0.53	0.912
PH	145.49±4.69	143.64±4.44	78.14±2.78	78.82±3.65	16.27	0.001

SE: standard error; RL1: root length of 15 days seedling; RL2: root length of 30 days seedling; RL1: Lateral root of 15 days seedling; RL2: lateral root of 30 days seedling; RM1: root mass of 15 days seedling; RM2: root mass of 30 days seedling; B/P: branches plant^{-1}; P/P: pods plant^{-1}; PW/P: pod weight plant^{-1}; S/P: seeds pod^{-1}; 100SW:100 seed weight; GY/P: grain yield plant^{-1}; PB: plant biomass; PH: plant height.

Table 4. Pearson correlation coefficients between root architecture and yield contributing traits of 15 and 30 days plants.

Traits	RL1	RL 2	LR1	LR2	RM1	RM2
Branches/ plant	-0.028	-0.046	-0.059	-0.017	-0.05	-0.096
Pod/ plant	0.203	0.167	0.581**	0.634**	0.321	0.215
Pod weight/plant	0.029	0.026	0.409*	0.467*	0.267	0.133
Seed/ pod	-0.018	-0.096	0.262	0.319	0.246	0.108
100 Seed weight	0.447*	0.495*	0.226	0.231	0.556**	0.550**
Grain yield/ plant	0.067	0.038	0.425*	0.471*	0.261	0.129
Plant biomass	0.168	0.135	0.461*	0.514*	0.309	0.247
Plant height	0.411*	0.443*	0.203	0.116	0.407*	0.505*

** Correlation is highly significant at the 0.01 level and * Correlation is significant at the 0.05 level

RL1= root length of 15 days old seedling; LR2= root length of 30 days old seedling; LR1= lateral root of 15 days old seedling; RL2= lateral root of 30 days old seedling; RM1= root mass of 15 days old seedling and RM 2= root mass of 30 days old seedling.

Genetic Linkage of root architecture with YCT

Cluster analysis

To estimate the genetic linkage of root architecture (Fig 3 and 4) with YCT, eight quantitative traits were scored in the present study. Cluster analysis separated 24 pea lines into four clusters, shared a common node at the similarity coefficient of 80%. It was observed that the lines grouped in the cluster-I were all tall with long roots. While the lines in cluster-II were tall with short roots. Similarly, the lines of cluster-III were dwarf with long roots and in cluster-IV, dwarf lines with short root were grouped (Fig. 5).

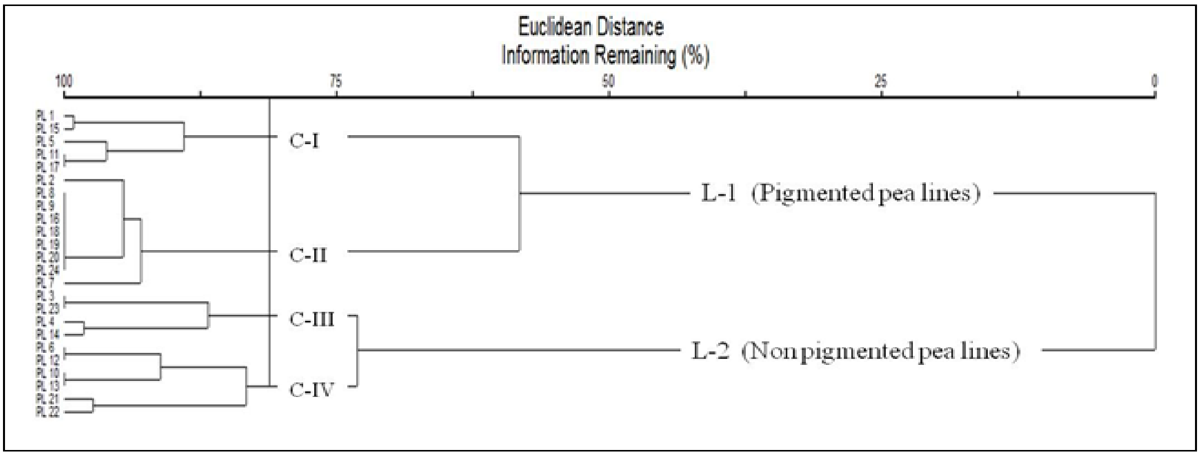

Fig. 1. Genetic linkage of anthocyanin pigmentation with seed phenotype in 24 Novel hybrid pea lines.

C-I: Brownish seed coat with wrinkled seed

C-II: Brown seed coat with wrinkled seed

C-III: Creamy seed coat with round seed

C-IV:Creamy seed coat with wrinkled seed.

Frequency distribution revealed that 79% in tall lines were long rooted, while 21% were short rooted plants. Likewise, 57% had a high number of lateral roots while 43% had a low number of lateral roots and 71% had high root biomass while 29% had low root biomass. Whereas, in dwarf lines, 40% were long rooted and 60% were short rooted plants, 57% had a high number of lateral roots while 43% had a low number of lateral roots and 36% had high root biomass while 64% had low root biomass as represented in Table 2. The genetic linkage of root architecture with YCT was reconfirmed through

Scatter Plot (Fig. 6). The plot also identified the same genetic association among 24 pea lines.

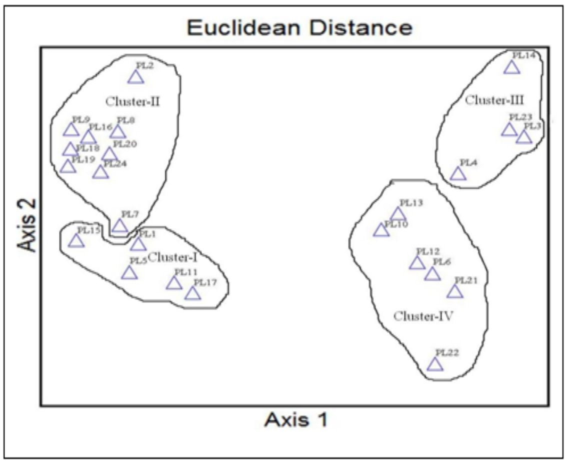

Fig. 2. The validation of genetic linkage based on anthocyanin pigmention on different part of the plant body and seed phenotype, extracted from Euclidean distance. Cluster-I representing pea line with brownish seed coat and wrinkled seed, cluster-II represent pea line with brown seed coat and wrinkled seed, cluster-III represent pea lines with creamy seed coat and round seed while cluster-IV represent pea lines with creamy seed coat with wrinkled seed.

Kruskal Wallis test

From the cluster analysis four clusters (Cluster I - IV) were identified, summary of the eleven quantitative traits of different clusters are represented in Table 3. Pea lines with long root and high root biomass were grouped in cluster-I. While short rooted plants with low root mass were grouped in a cluster-II. Similarly lines with maximum number of lateral roots were grouped in cluster-III. While lines with minimum number of lateral roots were grouped in cluster-II. Whereas cluster-III and cluster-IV grouped pea lines which were contributed intermediate values for root length and biomass. Likewise, cluster-I and cluster-IV grouped pea lines which were contributed intermediate values number of lateral root emergence. Statistical analysis of the Kruskal Wallis test shows a high degree of genetic diversity for root phenotypic traits and plant height. There were significant differences related to root length, root biomass, number of lateral roots and plant height among clusters, at the probability $P < 0.05$ (Table 3).

Fig. 3. Phenotypic variation in root architecture and plant height in 15 days old seedling of 24 novel hybrid pea lines.

Pearson correlation

The correlation between root architecture and yield contributed traits were analyzed using Pearson's correlation coefficient (Table 4). It was calculated that root length of 15 and 30 days old plants was significantly correlated ($P \leq 0.05$) with 100 seed weight (mean $r = 0.47$) and plant height (mean $r = 0.43$). Similarly, a number of lateral root emergency was significantly correlated with pod weight plant^{-1} (mean $r = 0.44$), grain yield plant^{-1} (mean $r = 0.45$) and plant biomass (mean $r = 0.49$). While, a number of lateral root emergency was highly significantly correlated ($P \leq 0.01$) with number of pod plant^{-1} (mean $r = 0.61$). Root biomass were highly significantly correlated with 100 seed weight (mean $r = 0.55$) and significantly correlated with plant height (mean $r = 0.46$).

Fig. 4. Phenotypic variation in root architecture and plant height in 30 days old seedling of 24 novel hybrid pea lines.

Discussion

The genetic association of various traits in legumes, provides an opportunity for improvement of economic traits (Sultana *et al.*, 2005; Akhtar *et al.*, 2011; Asim, 2012). However, the understanding of genetic linkage among the different traits is essential for the development of varieties with favorable combination of traits that improve the acceptability of pea. Morphological traits and appropriate statistical methods such as cluster analysis, principal component analysis (PCA) are useful tools for assemblage accessions and assigning them into respective gene pools (Burle *et al.*, 2011). Cluster analysis is a reliable method for identifying homogeneous groups of plants (Mooi and Sarstedt, 2011).

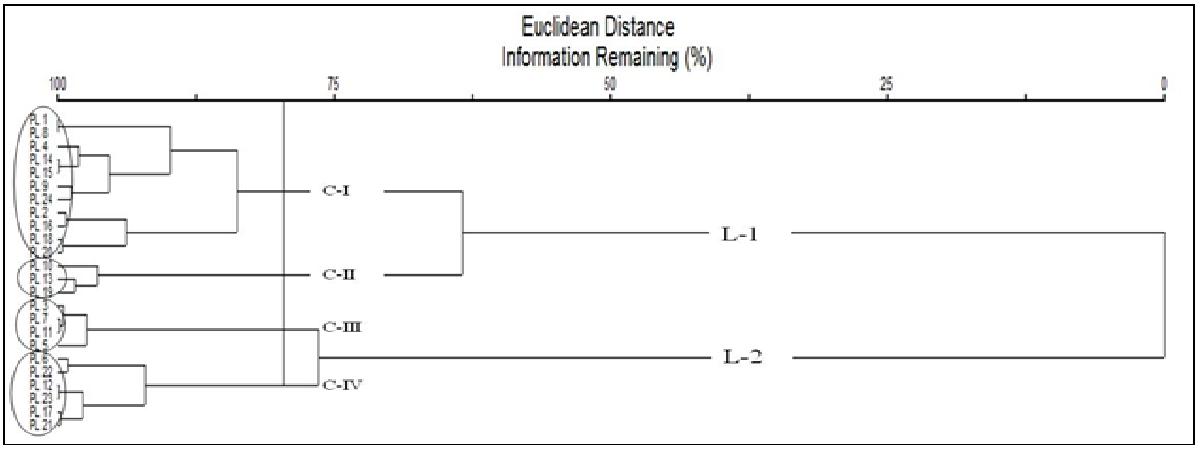

Fig. 5. Ward's agglomerative cluster analysis of root architecture and YCT, based on Euclidean Pythagorean distance measure

C-I: Tall plants with long roots: 79%

C-II: Tall plants with short roots: 21%

C-III: Dwarf plants with long roots: 40%

C-IV:Dwarf plants with short roots: 60%.

In the present study, multivariate analysis cut-apart 24 hybrid pea lines into 2 lineages based on the presence and absence of anthocyanin pigmentation. It was found that seed coat pigmentation showed strong correlation with the presence of pigmentation on flower and leaf axils. While, the absence of pigmentation of seed coat was always associated with white flowers and leaf axils with no pigmentation.

This finding indicates that the allele of a gene responsible for the presence or absence of anthocyanin pigmentation on different part of the plant body independently inherit in the pea population (Reid and Ross, 2011 and Egbadzor 2012). It was also observed that the seed shape (round or wrinkled) showed no correlation with the white flower and white leaf axils. The result signifies that the allele of a gene responsible for seed shape independently inherit despite the consequences of white flower colour (Haddis Yirga and Dargie Tsegay, 2013). In long this linked groups is very helpful for the plant breeders as indicators for the confirmation of crosses even in the F-1 generation (Nisar *et al* 2009).

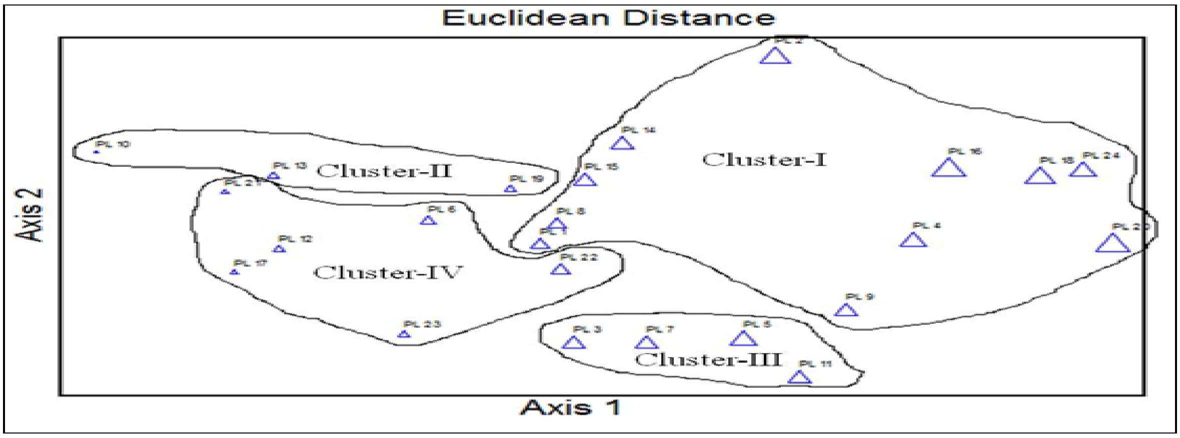

Fig. 6. The validation of linkage based on root architecture and YCT, extracted from Euclidean distance. Cluster-I representing tall pea lines with long roots, cluster-II represent tall pea line with short roots, cluster-III represent dwarf pea lines with short roots and cluster-IV represent dwarf pea lines with short roots.

The relationship between root related traits and yield contributed traits are more complex. However the balance between root traits and yield traits is of significant meaning for plant growth (Lynch, 2007). Result of the study obtained from cluster analysis and scatter plot was parallel. This finding indicate that the root architecture has close association with yield contributed traits. The results of the several studies pointed out that root system is highly correlated with yield and yield contributed traits by Kumar *et al.*, (2012) in *lens esculentus*, Kashiwagi *et al.*, (2006) in *Cicer arietinum*, Daur *et al.*, (2011) in *Vicia faba*. The result of the present research showed that plant height had the significant positive correlation with root length, and root biomass while plant height had no significant genetic association with lateral roots. Similarly, Jensen (1985) studied the root traits of pea and examined that root length and plant height are highly correlated. In rice, long root system is highly correlated with high yielding varieties (Kanbar *et al.*, 2009; Abd Allah *et al.*, 2010). In the present study, it was clear observed that the root length is significantly correlated with yield related traits (100 seed weight). Parallel results were also made by Jin *et al.* (2010), who founded that high yielding *Glycine max* genotypes have greater root length. The results of Person's correlation showed that number of lateral roots are significantly and positively correlated with pod plant^{-1}, pod weight^{-1} plant, seeds pod^{-1}, plant biomass and plant yield production plant^{-1}. Similar results were also made by Lamb *et al.* (2000), who pointed out that alfalfa genotypes with higher number of lateral roots had a greater total herbage yield. Furthermore, positive and significant correlation between lateral roots and yield production were observed by Sarker *et al.* (2005) in lentil. The present results also showed that root length was not correlated with pod weight plant^{-1}, seed number pod^{-1}, number of pods per plant and seedling stem biomass. This is in agreement with previous observations which pointed out the lack of correlation between root length and number of seed pod^{-1}, number of pods plant^{-1} and the mean number of seed plant^{-1} (Androsiuk, 2012).

Conclusion

This study suggested that the alleles of a gene, controlling seed coloration are coherently circulating in the pea lines with the alleles of a gene responsible for anthocyanin pigmentation. While the gene responsible for white flower is circulating in pea lines independently regardless of seed shape (round or wrinkled). Furthermore, it was found that root morphological traits have significant correlation with yield contributing traits (plant height, number of pods per plant, pod weight, grain yield and total biomass). Based on the suggested correlations, the plant breeder could utilize the root morphological traits for plant breeding program.

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