GENETIC STUDIES FOR IMPROVING SEED YIELD AND QUALITY TRAITS INCLUDING CAROTENOIDS, CHLOROPHYLL AND PROTEIN CONTENTS IN PEA (*PISUM SATIVUM* L.)

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Abstract

Among pulses, pea crop has gained a great interest as a protein source, which is essential for human nutrition and for animal feed. It contains carbohydrates, minerals and vitamins, especially vitamin B complex. In Pakistan, pea production is low as compared to other countries which can be attributed to lack of high yielding genotypes, besides other factors. The present study was conducted to evaluate the potential of pea crop for enhanced yield and quality. For this purpose eight accessions namely 19605, 20202, 26710, 20300, 19716, 20126, 26701 and 20153 were (selected from 99 accessions) crossed in half diallel fashion. Parents, F_1 and F_2 population were field planted and data were collected for yield and quality related traits i.e. carotenoids, chlorophyll and protein contents. Significant genotypic differences were observed for all the traits. Specific combining ability variance was found higher as compared to general combining ability variance in both F_1 and F_2 generations, which indicated the importance of non additive type of gene action in the inheritance of these traits. Accession 20126 was found good general combiner for seed yield per plant and carotenoids contents in F_1 generation, while accession 20153 was observed as good general combiner for seed yield per plant in F_2 generation. Accession 26710 was recorded as a good general combiner for total proteins in F_1 generation, while accession 20126 was found good general combiner for total proteins in F_2 generation. Cross combination 20202×26710 was good specific combination for seed yield per plant in F_1 generation while cross combination 19605×20202 was found best combination for seed yield per plant in F_2 generation due to maximum significant positive specific combining ability effects. Cross combination 20300×20153 was found best combination for total proteins in F_1 generation while in F_2 generation 19605×19716 was best cross combination for total proteins. In conclusion, the tested parental genotypes possessed favorable genes for most of the studied traits that can be utilized in breeding programs to improve garden pea.

Key words: Combining ability, Pea, Quality traits, Seed yield.

Introduction

Pulses are important leguminous crops, which are not only grown for protein purpose but also for carbohydrates, minerals and vitamins especially vitamin B complex (Kumar *et al*., 2017). Among pulses, garden pea (*Pisum sativum* L.) is one of the most important rabi crop grown as garden and field crop throughout temperate regions of world (Khan *et al*., 2017).While it is second important food legume crop as well as popular pulse crop in the world. As a protein source, pea crop has considerable position for human nutrition as well as for animal feed because it contains 21-33% protein contents both in pods and seeds (Santalla *et al.*, 2001).

In Pakistan, pea is cultivated on 21.620 thousand hectares with 139.233 thousand tons production (MNFSR, 2015). It is cultivated in the plains as well as in highlands of Pakistan during winter and summer, respectively but unfortunately average yield is poor (tons/ha) as compared to other countries (Nazir *et al.,* 1994). There are many factors for low yield in Pakistan but the main reason is availability of low genetic variability due to limited number of parents, which can be used in breeding programs (Baranger *et al.,* 2004).

Genetic diversity can be regarded as a baseline for improvement of crop plants in any breeding programs (Jarwar *et al.,* 2019). Genetic improvement of crop plants can be achieved either through selection or by development of hybrids provided knowledge about additive and non-additive type of gene action known for most of the economic traits. Griffing (1956) approach of diallel analysis facilitates breeders with detailed information about genetic systems as well as gene action towards the expression of most economic traits in the available germplasm. It also facilitates breeders to identify superior parents as well as best cross combinations on the basis of general and specific combining ability with respect to target trait that can be utilized in future breeding programs. Earlier, Singh & Singh, 2003; Patel, 2012 have reported that specific combining ability variance was found more as compared to general combining ability variance for protein contents, thus depicting the role of non additive type of gene action in the inheritance of proteins contents in *Pisum sativum* L. Singh *et al.,* 2010; Punia *et al.,* 2011 reported additive type of gene action towards the expression of seed yield related traits in *Pisum sativum* L. There is a need to study genetic basis of variation of seed yield and quality related traits in the available germplasm for development of high yielding varieties.

Association studies between seed yield and quality traits can be better understood by correlation coefficient. The nature and magnitude of correlation coefficient help the breeders to determine the selection criteria (Nisar *et al.,* 2008). Correlation analysis is helpful in breeding of plants for cultivar development (Kumar *et al.,* 2003; Kumar & Sharma, 2006). When direct selection becomes inapplicable, correlation analysis help plant breeders to improve the studied trait through indirect selection. Earlier researchers Kielpinski & Blixt (1982); Karjalainen

& Kortet (1987) reported negative correlation between grain yield and seed protein in pea. There is a need to find out the relationship between seed yield and quality related traits of the available germplasm.

Keeping in view the importance of pea crop, this research work was conducted to identify potential parents and cross combinations to be used in further breeding programs for improvement of seed yield and quality.

Materials and Methods

The present research was carried out in the vegetable section of department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, that is situated at an altitude of about 184 meters from sea level having coordinates 31.45° N and 73.13° E. The experimental material was comprised of 99 accessions of *Pisum sativum* L. collected from National Agricultural Research Centre (NARC), Islamabad Pakistan. Germplasm was planted under randomized complete block design using three replications during winter season 2013. Plant to plant distance was kept 15 cm while row to row distance was 30 cm. All other agronomic measures were kept constant throughout the season. The data were recorded for chlorophyll contents, carotenoids contents, total proteins and seed yield per plant. Eight diverse pea genotypes (accession # 19605, 20202, 26710, 20300, 19716, 20126, 26701 and 20153) were selected on the basis of multivariate analysis (data not shown). The seeds of selected genotypes were sown during winter season 2014. At the time of flowering, eight genotypes were crossed in half diallel fashion (Griffing, 1956) excluding reciprocals. Twenty eight crosses and eight parents were planted in field during 2015 under randomized complete block design in three replications. Each genotype was planted in single row containing ten plants. Row × row distance of 30 cm and plant × plant distance of 15 cm was kept constant. Each F_2 generation was planted in field ten rows in winter season during 2016. Each row contained fifteen plants. All genotypes were planted under randomized complete block design in three replications. Plant to plant and row to row distance was kept same as in F_1 generation. The observations were recorded on the basis of ten randomly selected plants from each genotype in each replication for chlorophyll contents (mg/g fresh wt.) and carotenoids contents (mg/g fresh wt.), which were measured by Arnon (1949) and Davies (1976) method and total proteins (%) were measured by Chapman & Parker (1961) method. Using digital electronic balance of OHAUS-GT400, USA

seed yield was weighed separately from each plant and measured in grams.

Statistical analysis

Data were subjected to analysis of variance (Steel *et al.,* 1997) to find out the variability among genotypes. General and specific combining ability effects were estimated following Griffing (1956) Model I, Method II. Correlation between seed yield, chlorophyll contents, carotenoids contents and total proteins was estimated for F_2 generation by Pearson (1920) method.

Results

Diallel analysis of variance: The analysis of variance for combining ability was carried out for all the studied traits in F_1 and F_2 generations of *Pisum sativum* L. which showed highly significant results for chlorophyll contents, carotenoids contents, total proteins and seed yield per plant (Table 1). Mean square due to general combining ability was found higher as compared to mean square for specific combining ability for chlorophyll contents, total proteins and seed yield per plant in *Pisum sativum* L. in F_1 generation, while in F_2 generation mean square for general combining ability was found higher for chlorophyll contents, carotenoids contents and total proteins in pea genotypes. For F_1 and F_2 generations of *Pisum sativum* L. components of variation showed that specific combining ability variance was higher as compared to general combining ability variance for all the studied parameters. Ratio due to general combining ability variance to specific combining ability variance was found less than one for all the studied traits in both generations.

Correlation analysis: Correlation analysis was performed among chlorophyll contents, carotenoids contents, total proteins and seed yield per plant to find out association between these studied traits in *Pisum sativum* L. Positive and significant correlation was found between carotenoids contents and total proteins in *Pisum sativum* L. (Table 4). Negative correlation was observed between chlorophyll contents and total proteins. While, positive and non-significant correlation was find out between chlorophyll contents and carotenoids contents; chlorophyll contents and seed yield per plant; carotenoids contents and seed yield per plant as well as total proteins and seed yield per plant.

Table 1. Diallel analysis of variance for yield and quality related traits in F_1 and F_2 generations of *Pisum sativum* L.

Source of variation	Chlorophyll contents (mg/g fresh wt.)		Carotenoids contents (mg/g fresh wt.)		Total proteins (%)		Seed yield per plant (g)	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
GCA	0.29**	0.20**	1.47**	2.69**	18.10**	13.04**	15.70**	7.84**
SCA	0.10**	0.10**	1.57**	0.46**	11.47**	7.86**	11.13**	9.52**
Error	0.001	0.009	0.25	0.09	0.14	0.21	0.35	0.42
δ^2_{GCA}	0.02	0.01	0.12	0.25	0.11	1.28	1.53	0.74
δ^2_{SCA}	0.09	0.09	1.32	0.37	0.34	7.65	10.77	9.10
$\delta^2_{GCA}/\delta^2_{SCA}$	0.29	0.20	0.09	0.68	0.15	0.16	0.14	0.08

Table 2. General combining ability effects for yield and quality related traits from eight *Pisum sativum* L. genotypes.

Parents	Chlorophyll contents (mg/g fresh wt.)		Carotenoids contents (mg/g fresh wt.)		Total proteins (%)		Seed yield per plant (g)	
	F₁	F₂	F₁	F₂	F₁	F₂	F₁	F₂
19605	-0.28**	-0.17**	0.46**	-0.63**	-1.54**	-0.07	-1.43**	-1.63**
20202	-0.09**	0.06*	-0.18	-0.65**	0.53**	-0.95**	-0.03ns	0.53**
26710	-0.01ns	0.08**	-0.04	-0.06	1.65**	-1.20**	-1.57**	-0.27ns
20300	-0.08**	-0.28**	0.09	-0.26**	0.58**	-1.23**	-0.07ns	0.70**
19716	-0.05**	0.14**	0.12	-0.06ns	-0.74**	0.45**	1.60**	-0.27
20126	0.10*	0.05	0.52**	0.74**	1.08**	1.87**	1.67**	0.50*
26701	0.28**	0.04	-0.44**	0.58**	0.65**	1.25**	-0.83**	-0.67**
20153	0.14**	0.07*	-0.54**	0.33**	-2.20**	-0.12	0.67	1.10
S.E.	0.01	0.02	0.15	0.08	0.13	0.11	0.19	0.58

Table 3. Specific combining ability effects for yield and quality related traits in 8 parents half diallel cross in F₁ and F₂ generation of *Pisum sativum* L.

Cross combinations	Chlorophyll contents (mg/g fresh wt.)		Carotenoids contents (mg/g fresh wt.)		Total proteins (%)		Seed yield/plant (g)	
	F₁	F₂	F₁	F₂	F₁	F₂	F₁	F₂
19605×20202	-0.11**	-0.16**	0.34ns	0.41**	-0.59**	2.44**	1.54**	4.21**
19605×26710	-0.09**	-0.07ns	1.54**	0.82**	-6.71**	1.45**	-1.93**	-0.99**
19605×20300	0.28**	-0.01ns	-1.26**	-0.97**	-4.64**	0.49**	1.24**	-5.96**
19605×19716	0.04**	-0.23**	-0.63**	-0.18ns	3.01**	3.64**	-5.09**	-2.99**
19605×20126	-0.27**	0.12**	-0.70**	1.36**	3.19**	0.05ns	1.51**	-2.09**
19605×26701	0.05**	0.33**	0.60**	0.51**	3.96**	1.84**	-0.33ns	0.08ns
19605×20153	0.35**	0.61**	2.04**	-1.40**	2.14**	1.54**	-5.49**	2.98**
20202×26710	0.11**	0.10*	0.17ns	-0.30*	2.23**	0.84**	5.34**	-2.82**
20202×20300	0.28**	0.26**	1.37**	0.61**	0.63**	1.54**	-1.16**	2.88**
20202×19716	-0.05**	-0.03ns	1.67**	-0.23ns	-0.72**	1.52**	-4.49**	3.18**
20202×20126	0.34**	-0.35**	0.27ns	-0.02ns	1.79**	-3.63**	-0.56*	1.08**
20202×26701	0.32**	-0.39**	-0.76**	-0.07ns	3.23**	0.72**	3.94**	-2.09**
20202×20153	-0.54**	-0.40**	-1.00**	0.42**	-0.59**	0.09ns	-4.23**	-4.52**
26710×20300	0.34**	-0.16**	-1.76**	0.09ns	2.18**	1.79**	-3.63**	1.68**
26710×19716	0.50**	-0.18**	-0.13ns	0.88**	1.99**	2.10**	3.37**	1.98**
26710×20126	-0.31**	0.18**	1.14**	-0.01ns	2.34**	-3.98**	3.97**	2.54**
26710×26701	-0.63**	0.42**	0.44*	-0.56**	3.44**	-5.70**	-1.19**	-4.29**
26710×20153	0.01ns	0.43**	-0.13ns	-0.21ns	-1.04**	-3.33**	0.31ns	2.61**
20300×19716	-0.03ns	-0.39**	1.07**	-0.51**	0.06ns	1.80**	1.87**	3.01**
20300×20126	0.10**	-0.16**	0.34ns	-0.67**	2.24**	-2.95**	-6.19**	-1.42**
20300×26701	0.24**	-0.04ns	2.30**	0.28*	4.34**	0.34ns	1.97**	0.41ns
20300×20153	-0.42**	0.15**	-0.93**	0.87**	4.69**	-4.63**	-0.53*	-2.69**
19716×20126	0.06**	0.35**	-1.36**	-0.34**	-5.27**	-2.30**	0.14ns	-0.12ns
19716×26701	0.18**	0.36**	0.94**	-0.89**	-1.51**	-1.35**	1.31**	2.04**
19716×20153	0.28**	0.33**	1.70**	0.83**	-0.99**	3.02**	3.14**	2.28**
20126×26701	-0.33**	-0.02ns	-0.13ns	0.25*	-1.32**	0.24ns	0.24ns	1.94**
20126×20153	-0.09**	-0.41**	-1.36**	-1.13**	-4.14**	3.27**	0.74**	-5.49**
26701×20153	0.22**	-0.29**	-1.06**	-0.25*	-3.04**	0.22ns	1.91**	1.01**
S.E.	0.01	0.03	0.20	0.11	0.15	0.18	0.23	0.25

Table 4. Correlation coefficients for yield and quality traits in F₂ population of *Pisum sativum* L.

	CC	TP	SYPP
ChlC	0.130	-0.118	0.119
CC		0.303**	0.154
TP			0.035

ChlC= Chlorophyll contents, CC= Carotenoids contents, TP= Total proteins and SYPP= Seed yield per plant

General combining ability effects: The highest positive and significant values of general combining ability effects are considered desirable for chlorophyll contents, carotenoids contents, total proteins and seed yield per plant. In F₁ generation, accession 26701 was good general combiner followed by accession 20153 and accession 20126 due to maximum and positive general combining ability effects for chlorophyll contents (Table 2). For carotenoids contents accession 20126 was good general combiner followed by accession 19605 due to maximum and positive general combining ability effects, while for total proteins accession 26710 was good general combiner followed by accession 20126 and accession 26701 due to positive general combining ability effects. Accession 20126 was good general combiner for seed yield per plant followed by accession 19716 and accession 20153. While in F₂ generation accession 19716 was good general combiner followed by accession 26710 and accession 20153 for chlorophyll contents due to positive general combining ability effects. For carotenoids contents, accession 20126 was good general combiner followed by accession 26701 and accession 20153 due to positive general combining ability effects. Accession 20126 was good general combiner for total proteins followed by accession 26701 and accession 19716 due to positive general combining ability effects. For seed yield per plant, accession 20153 was good general combiner followed by accession 20300 and accession 20202 (Table 2).

Specific combining ability effects: For chlorophyll contents maximum positive and significant specific combining ability estimates were observed by cross combinations 26710×19716 followed by 19605×20153 and 20202×20126 in F₁ generation and are good specific combinations (Table 3). For carotenoids contents cross combination 20300×26701 followed by cross combinations 19605×20153 and 19716×20153 were good specific combiners due to positive and significant specific combining ability effects. Cross combination 20300×20153 exhibited good specific combining ability followed by cross combinations 20300×26701 and 19605×26701 for total proteins due to positive and significant specific combining ability effects. For seed yield per plant, cross combination 20202×26710 showed good specific combining ability followed by cross combinations 26710×20126 and 20202×26701 due to positive and significant specific combining ability estimates in F₁ generation. While in F₂ generation, for chlorophyll contents good cross combinations were observed by 19605×20153 followed by 26710×20153 and 26710×26701. For carotenoids contents cross combination 19605×20126 was good specific combination followed by cross combination

26710×19716 and cross combination 20300×20153. Cross combinations 19605×19716 was good specific combination for total proteins followed by cross combinations 20126×20153 and 19716×20153. For seed yield per plant cross combinations 19605×20202 was good specific combination followed by cross combinations 20202×19716 and 20300×19716 due to maximum positive and significant specific combining ability estimates (Table 3).

Discussion

Legumes are second important crop plants after grass family because these are major source of plant based proteins as well as significant amount of carbohydrates, minerals and vitamins. In Pakistan pea is cultivated in both plain and hilly areas. But, the production of pea is not enough to fulfill food and export requirements due to low yield and poor quality of seed. For crop improvement, knowledge about genetic diversity among crop species is the primary requirement. Statistical analysis of various traits related to seed yield and quality displayed significant amount of genetic variability in both generations. Earlier researchers like Sureja & Sharma, 2000; Amjad & Anjum, 2002; Nisar *et al.,* 2008; Tiwari & Lavanya, 2012; Pal & Singh, 2013; Kamdi *et al.,* 2015; Khan *et al.,* 2017 have documented genetic variability for seed yield and its related traits in *Pisum sativum* L. When such a significant and valuable genetic variability is available for selection, knowledge about relative contribution of additive and non additive type of gene action for the trait of interest is important before selection to improve the trait. Diallel mating design is one the best methods to estimate additive and non additive types of gene actions as well as general and specific combining ability effects (Srivastava *et al.,* 2000; Bourion *et al.,* 2002).

Analysis of variance for both generations (F₁ and F₂) demonstrated significant amount of general combining ability and specific combining ability in the inheritance of studied traits indicating the importance of additive and non additive type of gene action in *Pisum sativum* L. Earlier reports by Singh *et al.,* 2010; Abbas, 2012; Dagla *et al.,* 2013; Mishra *et al*., 2014; Kumari *et al.,* 2015; Chouhan *et al.,* 2016; Kumar *et al.,* 2017; Shivaputra*et al.,* 2018 demonstrated presence of additive and dominant gene effects in the inheritance of seed yield and quality traits in *Pisum sativum* L. In F₁ generation chlorophyll contents, total proteins and seed yield per plant showed more general combining ability effects as compared to specific combining ability effects, while in F₂ generation chlorophyll contents, carotenoids contents and total protein displayed more general combining ability effects indicating contribution of additive type of gene action.

Selection of these traits may be effective to develop promising pea hybrids or OPV. Earlier reports by Sachan *et al.,* 2001; Singh *et al.,* 2010; Punia *et al.,* 2011 documented additive type of gene action in the inheritance of seed yield and its related traits in pea crop.

In F_1 generation carotenoids contents showed more specific combining ability effects than general combining ability effects while in F_2 generation seed yield per plant showed more specific combining ability effects displayed the importance of dominance type of gene action in the inheritance of these traits that suggested delay selection for breeding of stable yielding and good quality pea genotypes. Singh & Singh, 2003; Ceyhan *et al.,* 2008 reported dominance type of gene action in the inheritance of seed yield related traits in *Pisum sativum* L.

The accession 20126 was found good general combiner for carotenoids contents and seed yield per plant in F_1 generation while the same accession was found good general combiner for carotenoids contents and total proteins in F_2 generation of *Pisum sativum* L., due to maximum positive and significant general combining ability effects. For chlorophyll contents accession 26701 was found good general combiner in F_1 generation while accession 19716 was found good general combiner for chlorophyll contents in F_2 generation due to maximum positive and significant general combining ability effects. Dagla *et al.,* 2013; Kumari *et al.,* 2015; Sharma *et al.,* 2015; Chouhan *et al.,* 2016 demonstrated that significant general combining ability effects in positive direction for seed yield were due to similar general combining ability effects of some seed yield related traits. That indicates that combing ability of seed yield was influenced by combined effect of these seed yield related traits. So, simultaneous improvement of these seed yield related traits with seed yield may be utilized to raise yield potential in pea genotypes.

In F_1 generation 20202×26710 was considered the best cross combination for seed yield per plant, interestingly both parents were poor combiner for the trait. While in F_2 generation accession 19605 had minimum general combining ability effects for seed yield per plant but when it combined with accession 20202 which was good general combiner for the said trait, proved to be the best cross combination for seed yield per plant. A plenty of reports have been documented involving good × good, good × poor and poor × poor parents that results in outstanding performing hybrids for the trait of interest (Imran *et al.,* 2012). For total proteins 20300 × 20153 was considered best cross combination involving parents of good and poor general combining ability in F_1 generation respectively while, in F_2 generation accession 19605 was considered poor combiner for total proteins but when it combined with accession 19716 that was good combiner for the studied trait, it proved to be the best cross combination for total proteins. Earlier reports by Mishra *et al.,* 2014; Sharma *et al.,* 2015; Chouhan *et al.,* 2016; Shivaputra *et al.,* 2018 demonstrated significant and positive specific combining ability effects for seed yield and seed yield related traits in *Pisum sativum* L. may be responsible for development of high yielding pea genotypes.

Correlation analysis revealed that carotenoids contents had significant and positive correlation with total proteins in *Pisum sativum* L. depicted that simultaneous improvement of these two traits can be possible. The trends of these results were in accordance with Reichert & Mackenzie (1982). They recorded positive correlation between carotenoids contents and total proteins.The studies indicated that the parental germplasm has potential for development of high yield varieties in pea with improved quality traits.

Conclusion

From the above discussion it can be concluded that cross combination 20202 × 26710 was a good specific combination for seed yield per plant due to highest positive and significant specific combining ability estimates and for total proteins due to moderate positive and significant specific combining ability estimates in F_1 generation. While in F_2 generation cross combination 19605 × 20202 can be considered as best cross combination for seed yield per plant in *Pisum sativum* L. due to highest positive and significant specific combining ability estimates and for total proteins with moderate positive and significant specific combining ability effects. These cross combinations can be utilized in future breeding programs for high yield with improved protein contents. In the present study, all the studied traits showed additive and non-additive type of gene action but the dominance of non-additive type of gene action towards the expression of said traits suggesting that heterosis breeding would be more rewarding for improvement of yield and selection can be delayed up to F_4 and F_5 generations for fixation of desirable genes.

Acknowledgement

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