

ASSESSMENT OF PATTERN OF GENETIC VARIATIONS ON DRY MATTER CONTENTS AND PROTEIN LEVELS IN DRY PEAS (*Pisum Sativum* L.) VARIETIES

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Abstract: Genetic and climatic factors create variations in recorded yield parameter as well as in the portion of protein of dry pea seed in whole. The nutritional quality of dry pea grain has huge impact on the development of peas plant stand. The acreage and yield for dry peas are decreased in Pakistan owing to knowledge lacking. The pea lines/genotypes are different in these factors of study i.e. seed growth and vigor strength at seven leaf stage of peas plants, these factors affects the end product yield obtained from the experiment. This current field and glass house study demonstrated the behavior pattern of eight (08) dry pea genotypes for seed vigor, grain growth indices and made association of traits in the studies with recorded yield trait as well as protein factor. In current experiment the highest growth vigor I (one) was recorded in dry peas advance line DP-09-08. Advance line DP-09-08 has recorded more shoot length as compared to comparing varieties. In the experiment results, variety DP-09-08 has the highest Vigor Index II. The variety DP-NO.267 recorded yield 3705 kg/ha, this value is 17.20 % and 32.30% higher than two advanced lines DP-02-15 and DP-03-15, respectively. The DP-09-08 has highest protein (22.70%) value along with highest Vigor Index II (two) and it has recorded yield (4940 kg/ha) equal to variety DP-NO.267. The variety DP-NO.267 has least protein (20.10%) value. The dry peas advance line named DP-04-15 showed least yield potential as well as low protein portion. Studies show a significant and positive association between two parameters including vegetative growth, bio mass. It also revealed significant and positive association in recorded Vigor Index I only. Nitrogen intake has positive and significantly association with these parameters bio mass, plant growth. Vigor index I has significant and positive association in greenhouse only. These results demonstrates that seed growth indices character did not express any significant association with dry pea recorded yield as well as protein factor, so more research oriented efforts required to evaluate genotypes for high yield potential and plant vigor for the aim to increase area and yield of *Pisum sativum* in Pakistan especially in the province Punjab.

Keyword: correlation; dry peas; genotypes; heritability; *Pisum sativum*; protein; yield.

INTRODUCTION:

Dry pea crop seed is an important source of vegetable protein (21-27%) for human. Proteins are the essential ingredients of our food and are considered to be building block of our body. Proteins constitute about 20 percent of our body weight and are derived from the dietary foods. Pulses are considered to be the cheapest and economic source of protein. However, the availability of pulses

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had declined very much. Dry pea plants have nodule system which fixes the atmospheric nitrogen in the soil and this enriched soil can be used for the supply of nitrogen to the next growing crop. Therefore, peas have great importance for the crop rotation reported by Siddika et al. (2013). Among the pulse crops, field pea or dry pea (*Pisum sativum* L.) belongs to family leguminoceae and is considered to be originated of Ethiopia, the Mediterranean and Central Asia. It is a nutritious and protein rich crop, mostly used for green and dry seeds. Hence, pea is categorized as vegetable type and field pea. The area of field pea in Pakistan is about 46.50 thousand hectares and average production of 662 kg/ha (Anonymous, 2016-17).

To meet out challenging demand of pulses it has become necessary to increase their production in the country. Field pea has high production potential under better agronomic management. Field pea, very much response to low soil pH and one/two irrigations hence, there is plenty of scope for its expansion. Relatively this crop does not have much problem of pest and diseases except powdery mildew, to which genetic resistance is available. There is urgent need to give farmers varieties which yield better even under average agronomic management. Dwarf types have greater potential under one or two irrigations. Hence, there is need to combine together desirable gene(s) from tall and dwarf types for evolving high yielding, disease resistant and widely adopted varieties. To fulfill the target, the information obtained on genetic variability of recorded yield and its attributing traits is essentially required. Hence, the present study has been started to generate basic information in relation to genetic improvement in seed yield.

Understanding of genetic variability and heritability of characters under improvement is essential and pre-requisite for launching any breeding programme to achieve the goal (Janaki et al. (2015). Genetic improvement in relation to grain yield and harvest index is major objective in this crop. However, yield is a complex character contributed by several morpho physiological traits. Hence, the knowledge relating genetic control of yield and its contributing traits is of need of hour for initiating an efficient selection scheme for selecting a superior desirable genotype. Further, the study of genetic variability heritability and genetic advance would provide estimates for deciding an efficient and effective breeding programme in this crop. Earlier experiments have shown that pulses are important for diversifying pea (Miller et al. 2015). Legumes enhance the soil fertility by fixing the atmospheric nitrogen (Herridge et al. 1995). Pulses are good nutrient supplement. Dry peas are the second food legume which is being cultivated around the world after common beans (Tar'an et al. 2004; Wang and Daun 2004). Dry peas provide high food value of balanced amino acid (Borowska, Zadernowski, and Konopka 1996). Peas are being grown for different uses including making etc. (Chen et al. 2004; McKenzie and Spooner 1999). Both factors including yield and protein of dry peas are affected by environment as well as genetic factors (Nikolopoulou et al. 2007; Wang et al. 2010). Dry peas crop vary according to different environmental conditions i.e.,

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rainfall, soil type and temperature (Acikgoz et al. 2009). Powell et al. (1984) found that changes occurred in growth of seed. The major leading factor of seed, particularly seed growth is climatic factor (Delouche 1980). Actually seed growth is a seed quality, which expresses the potential of seed. Seed vigor is an independent trait of physiological potential to germinate (Marcos Filho 2015). Good quality seeds cope up stress conditions available them in the field conditions. Dias et al. (2010) found that vigor of seed effects on initial crop growth. Seed vigor is crucial factor for potential of seed (Marcos Filho 2015). Following methods used for determination of seed vigor e.g. electrical conductivity test (Matthews and Bradnock 1967), accelerated aging test (Caseiro and Marcos Filho 2000), automated computer imaging test (Chiquito, Gomes Junior, and Marcos Filho 2012). The tests are based on seed growth evaluation which includes first count of the plant stand, seed vigor classification, vigor trait of the seedling i.e. length or dry weight (Marcos Filho 2015). Ali Khan and Youngs (1973) found significant variability in pea protein, which vary according to locations, cultivars, years. So, the need is a test which provides good results for yield. The basic aim of seed growth parameter determinant is identification of protein value, plant stand as well as dependent factor yield. Determination of *Pisum sativum* genotypes for early vigor and yield attributes. Variations in field pea genotypes bring significant variations in early growth, so expressing prominent and significant heritability in the recorded seed protein as well as of yield trait. Therefore, the required targets of this research experiment were (a) to determine the pattern of variations in pea genotypes comparing to early growth at seven leaf level in greenhouse conditions, (b) to determine the association of got results with the recorded field seed protein level as well as with trait yield (c) to assess the association of seed growth index observed in greenhouse conditions and incubation experiment with field dry peas experiment.

MATERIAL AND METHODS:

An experiment was demonstrated in field conditions at experimental area of Pulses Research Institute (PRI), Ayub Agricultural Research Institute (AARI), Faisalabad (Latitude: 31°25'00" N, Longitude: 73°04'59"E, elevation above sea level: 186 m = 610 ft). The soil at the experiment is loam (fine). Weather indicators, including precipitation indication and recorded average temperature during peas crop season is shown in Table 1. No chemical fertilizers were supplied in soil prior to seed growth. Treatments included eight genotypes of dry peas i.e. DP-09-08, DP-01-15, DP-02-15, DP-03-15, DP-04-15, DP-05-15, DP-10-15, and DP-NO-267. The Randomized Complete Block Design comprising three repeats used. The size of the experiment plot was 4m x 1.2 m and the distance among rows maintained one feet. Seed sown in the month of November, 2017. After 90 percent maturity, the plots of dry peas were harvested in the month of April, 2018. Threshed seeds weight was recorded for each plot. The grain protein was calculated simultaneously. For greenhouse study small amount seed sample was used. The greenhouse experiment was demonstrated at Ayub Agricultural Research Institute (AARI), Faisalabad. The collected soil

filled in the experimental pots; it was collected from top 15 cm soil. Experiment was demonstrated on randomized complete block design having six repeats. Seeds of dry peas crop were sown in the month of April, 2016 i.e. 10 seeds/sown. The seed pots were irrigated uniformly. All experimental pots obtained and get equal quantity of irrigation for whole season to ensure only genotypes to be variable factor. The greenhouse conditions were maintained at 16 hour light along with 8 hour dark period, temperature range at 21°C along with 18°C (day/night period), and relative humidity is 50 ÷ 5% for whole experiment duration. After Ten days of sowing, plants in each experimental pot were calculated to know the seedling emergence count parameter. At seven leaves level, again plants counted. Every single plant in a single experiment pot was up-rooted to determine the different traits like root height, shoot height/plant, biomass of shoot and root. Uprooted plant samples dried at temperature range of 65°C (for 4 days). The dried samples were ground to passed through a mesh screen of size 1-mm. Analyzed the samples to assess the total nitrogen. The total nitrogen up-take (gram per pot) was estimated by (multiplying the nitrogen estimated with the recorded dry matter sample)/100. In greenhouse experiment, the same sample seed was examined in an incubator apparatus to know the plant vigor index. In incubator, 15 seeds/genotype in repeats of four were maintained moist for 8 days, maintained temperature of 30°C. After drying samples at temperature 65°C determine the seedling plant vigor parameters, i.e., length (cm) of the seedling samples and dry weights (g) of seedling samples. Samples of ten seedlings randomly taken to estimate the desirable data. Plant vigor indices for greenhouse experiment and incubation experiment were estimated by using these formulae of renowned scientists (1, 2) (Abdul baki and Anderson 1973) as

Vigor Index I = Germination percentage (%) x seedling length (cm) (1)

Vigor Index II = Germination percentage (%) x seedling dry weight (g) (2)

STATISTICAL ANALYSIS:

Recorded data of all traits were analysed for analysis of variance (ANOVA). Means values were identified using scientist Fisher LSD method at $p < 0.05$ after analysis of variance calculated significant F value. Recorded Pearson correlation component between different pairs.

RESULTS AND DISCUSSION:

FIELD EXPERIMENT:

Recorded yield of advanced lines/genotypes varies from 3705-4401 kg/ha, obtained a mean yield value of 4492 kg/ha. A significant variation was seen among different dry pea genotypes depending on grain yield (Table 2). The variety DP-09-08 yielded significantly higher yield (4401 kg/ha) as compared to lines DP-05-15 and 10-15 ($p = 00.0285$). The yield of DP-09-08 was 17.20 and 32.30% higher as compared to comparable two lines, i.e., DP-03-15 and DP-No.267, respectively. Muhammed et al. (2016) reported that dry pea varieties showed good impact of better yield and stability parameters in temperate to semi arid climates. Grain protein (nitrogen was multiplied by a factor of 6.25) for different genotypes varies from 20.10 to 22.40%, but they were not

significantly different ($p = 0.3151$, Table 2), recorded a yield mean value of 21.70 %. The highest seed protein was obtained with genotype DP- 09-08, while lowest protein was achieved with highest yielding genotype. The results also depicted that genotype DP- 09-08 has higher yield potential (4401 kg/ha) similar to DP-05-15, along with highest protein (23.50%). Atta et al. (2004) reported that recorded protein values differs significantly among nitrogen contents of different genotypes of dry peas, this shows their genetic difference for variation in protein factor. Actually this variation between genotypes is the potential of genotypes to maintain nitrogen fixation mechanism. Yoneyama and Ishizuka (1982) found that during grain filling process symbiotically fixed nitrogen is shifting to growing plant. It demonstrated that total amount of nitrogen varies with location, year, within plants (Ali Khan and Youngs 1973) as well as with trait of maturity duration (Pandey and Gritton 1975). Seed nitrogen is dependent on grain nitrogen factor as well as grain carbohydrate (Cataldo et al. 1975). High protein in peas seeds were found to be related to very low carbohydrate levels in seeds (Singhal et al. 1989); water contents deficiency and high temperature have association with low seed yield and elevated protein in peas (Stoddard and Marshall 1990).

GREENHOUSE STUDIES:

In the green house experiment no significant difference was noted for recorded above and below ground biomass/plant, shoots and root height trait. Recorded average above and below ground biomass weights were 1.330, and 0.660 g, respectively. An average root and shoot length trait were recorded to be 17.430, and 19.800 cm, respectively. Same is the case with above and below ground biomass/plant trait, no significant differences were observed in total dried biomass/pot ($p = 0.2260$), obtained mean biomass of 16.9 g/pot. Total plant nitrogen up-take/pot did not vary among dry pea genotypes, the two advanced lines DP-10-15 and DP-05-15 proved the highest nitrogen in-take (0.740 and 0.710 g/ pot) respectively, bring more bio mass storage possessing longer shoots and root height. Plant Vigor Index I and plant vigor index II did not reveal significant variations (Table 2). The highest plant vigor index I was recorded with dry peas line DP-09-08 accompanied more shoot length (24.380 cm) as compared to competitor genotypes. The highest plant vigor index II was obtained from both genotypes DP- 08-09 and line DP-05-15; this is owing to their high bio mass and germination rates traits respectively. Result reveals that besides plant bio mass and plant height trait and seedling growth has prominent impact on plant vigor indices. Taweekul et al. (1998) found prominent variation in plant seedling growth trait among several seed lots of field pea genotypes. Earlier research (Hampton and Scott 1982) reported reduce seed yield from lower plant stand trait. The genotypes having high seed vigor index resulted from more seedling emergence trait. Dias et al. (2011) found that plants grown from high seed growth and intermediate seed growth possess best results adverse to weed growth; they also reduce weed dry mass accumulation and obtained excellent outcome and seed yield trait.

CORRELATIONS BETWEEN SEED VIGOR INDICES AND OTHER CHARACTERS:

A positive and significant association was depicted among incubation experiment and greenhouse experiment for biomass weight per seedling ($r = 0.440$, $p = 0.0031$) and Growth index I ($r = 0.420$, $p = 0.00770$). No significant relation for growth Index II was demonstrated between incubation studies as well as in greenhouse experiment ($r = 0.11$, $p = 0.4880$). In greenhouse experiment, correlation technique was done to determine the association between some variables e.g., biomass of plants, vigor indices of plants, with obtained yield trait and seed protein information got from field experiment (Table 4). A positive and significant association ($r = 0.7650$, $p < 0.0001$) was recorded between total biomass/plant and Vigor Index I of plants. Total nitrogen up-take revealed a positive and significant association with total biomass/plant and Vigor Index I /plant. As plant nitrogen up-take is the interaction of biomass /plant with plant nitrogen, showing the capacity of a plant for nitrogen storage. Plant nitrogen uptake was dependent on dry matter value. Takeda and Frey (1979) reported that the plants with more recorded dry matter value have a more grain yield /plant. Total plant dry matter value is one factors demonstrating grain yield value. Recorded results revealed significant and contrasting association between Vigor Indices and plant nitrogen in-take. Plant Vigor Index I revealed a positive association while vigor Index II revealed a negative association with total nitrogen up-take all single plants in a pot. A comparison of different parameters made with field studies of agronomic parameters of genotypes demonstrated in greenhouse experiment while incubation experiment did not demonstrated significant associations (Table 4 and 5). The association between recorded grain yield and protein value was also revealed to be non-significant ($r = 0.0560$, $p = 0.7128$). Al Karaki and Ereifej (1999) also reported a negative association effect between recorded seed yield trait as well as seed protein value. Ali Khan and Youngs (1973) reported non significant association between seed protein content as well as in recorded grain yield. They also reported that selection for genotypes having high protein would not link with lethal effects on the other characters. Henry et al (1995) demonstrated that the protein components possess negative association with seed yield. Total protein (multiplying of recorded yield with protein value) revealed a positive and significant association with both of recorded yield ($p < 0.00020$) as well as protein value ($p = 0.00010$).

CONCLUSIONS:

The dry pea genotypes differ significantly in protein value and grain yield. The genotype DP-09-08 possesses lowest protein value it revealed highest yield potential. The dry peas advanced line DP-03-15 possessing similar protein value having lowest yield potential. The genotype DP-05-15 maintained higher yield potential (at par with advance line DP-09-08) possessing highest protein part (23.30%), this association of yield and seed protein value varied among genotypes. This correlation did not reveal any association between obtained yield and

protein value. Correlation revealed non-significant association between yield and seed protein percentage (%). The experiment showed a positive and significant association between two traits i.e. Vigor Index I and biomass per plant in green house experiment only. The results confirm that plant vigor indices inferred in incubation studies and green house experiment did not reveal significant link with seed yield and protein value recorded from field experiment plants. Results indicate that concerted efforts are looked-for to unfold genotypes for quantitative as well as qualitative traits at the preliminary stage of plant development.

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Table.1 Cumulative monthly air temperature and average monthly rainfall during the dry peas growing season.

Month	Temperature(°C)	Rainfall(mm)
	Monthly average	Monthly average
October	31.8	21
November	27.3	T
December	20.6	16.4
January	17	4.8
February	16.1	6.8
March	28.8	16.1
April	36.3	9

Table.2 Evaluation of different field pea varieties for early growth and vigor indices in green house and field conditions.

Variety	Above ground bio-mass/p/ha (g)	Below ground bio-mass/plant (g)	Root length(cm)	Shoot length(cm)	Total bio-mass/pot (g)	Total N uptake/pot (g/pot)	Vigor index I	Vigor index II	Yield (kg/ha)	Protein content (%)
DP-09-08	1.20	0.60	16.38	24.38	16.38	0.60	4222	180.2	4401	22.7
DP-01-15	1.30	0.70	18.40	18.40	16.40	0.70	3300	178.00	4212	21.3
DP-02-15	1.34	0.65	17.60	21.60	17.60	0.65	3600	175.00	4608	21.4
DP-03-15	1.30	0.60	17.00	18.00	17.00	0.60	3189	170.11	4700	23.3
DP-04-15	1.30	0.71	16.09	18.09	16.09	0.71	3090	174.5	4400	20.1
DP-05-15	1.36	0.80	16.00	18.00	16.00	0.60	3121	180.11	4341	22.1
DP-10-15	1.40	0.74	19.00	20.00	18.00	0.74	3327	178.71	4122	21.2
NO.267(C)	1.5	0.50	19.00	20.00	18.00	0.60	3402	180.00	3705	20.1
Mean	1.3375	0.6625	17.43375	19.80875	16.93375	0.65	3406.375	177.0788	4492.375	21.525
p-value	0.7344	0.2730	0.2370	0.2570	0.2370	0.2430	0.1630	0.6190	0.0265	0.3250
LSD	NS	NS	NS	NS	NS	NS	NS	NS	660	NS

Table.3 Evaluation of different field pea varieties for vigor indices in incubator.

variety	Shoot length(cm)	Root length(cm)	Bio mass/seedling (g)	Total Bio mass (g)	Vigor index I	Vigor index II
DP-09-08	3.21	9.00	0.430	4.41	1122	180.2
DP-01-15	2.70	9.00	0.440	4.80	1018	178.00
DP-02-15	2.40	11.10	0.441	4.54	1231	175.00
DP-03-15	1.00	9.00	0.480	4.42	0989	170.11
DP-04-15	2.30	6.09	0.462	4.60	850	174.5
DP-05-15	2.50	7.00	0.470	4.21	0921	180.11
DP-10-15	00.80	8.05	0.460	4.30	0993	178.71
NO.267(C)	2.00	07.20	0.450	4.50	1080	180.00
mean	2.11375	8.305	0.454125	4.4725	1025.5	177.0788
p-value	0.0001	0.0041	0.1071	0.0001	0.0425	0.0521
LSD	0.71	1.61	NS	0.150	NS	NS

Table.4 Pearson correlation coefficients and p-value(in parenthesis) showing the association between selected measured parameters and level of significance for the dry peas varieties for studies done in green house and field studies.

Pearson correlation coefficient Prob @ r r l under H0:Rho = 0							
Variables	Total biomass	Vigor I	Vigor II	Total N uptake	Protein content	yield	Total protein
Total biomass	-	-	-	-	-	-	-
Vigor I	0.765 (@0.0001)	-	-	-	-	-	-
Vigor II	2.45 (0.0901)	0.170 (0.1741)	-	-	-	-	-
Total N uptake	0.321 (0.0321)	0.367 (0.0051)	-0.448 (0.0012)	-	-	-	-
Protein content	-0.121 (0.5121)	-0.115 (0.5319)	0.067 (0.6771)	0.051 (0.7113)	-	-	-
Yield	0.071 (0.5601)	0.230 (0.2131)	2.56 (0.1454)	-0.261 (0.1651)	-0.058 (0.7119)	-	-
Total protein	-0.019 (0.07501)	0.090 (0.5198)	0.254 (0.1811)	-0.153 (0.3951)	0.653 (0.0002)	0.711 (0.0001)	-

Total biomass, vigor indices, and total N uptake were obtained from green house. Protein content, yield and total protein were obtained from field. Bold values represent significant values at p < 0.05.

Table.5 Pearson correlation coefficients and p-value(in parenthesis) showing the association between selected measured parameters and level of significance for the dry peas varieties for studies done in incubator and field studies.

variables	Total biomass	Vigor I	Vigor II	Protein content	yield	Total protein
Total biomass	-	-	-	-	-	-
Vigor I	0.015 (0.8771)	-	-	-	-	-
Vigor II	0.070 (0.5401)	0.170 (0.1741)	-	-	-	-
Protein content	-0.118 (0.3621)	-0.115 (0.5319)	0.067 (0.6771)	-	-	-
Yield	-0.071 (0.5501)	0.230 (0.2131)	2.56 (0.1454)	-0.058 (0.7119)	-	-
Total protein	-0.119 (0.3101)	0.090 (0.5198)	0.254 (0.1811)	0.653 (0.0002)	0.711 (0.0001)	-