



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; SPI: 1016-1021

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Evaluation of Seed Yield and Genetic Divergence in the Germplasms of *Pisum sativum* L. var. *arvense*

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Abstract

Keeping in view the prime objective of the plant breeding programmes is to make an improvement in yield of field pea (*Pisum sativum* L. var. *arvense*), the present investigation was undertaken to work out the direct and indirect effects of characters on seed yield by path coefficient analysis and by non-hierarchical Euclidean cluster analysis to study the genetic divergence among the one hundred twenty four field pea genotypes. Path coefficient analyses were estimated on phenotypic as well as genotypic levels to resolve the direct and indirect effects of different characters on seed yield of field pea. The overall review of the result obtained by genetic diversity study in present investigation revealed that the crosses between the entries separated by the large inter-cluster distances and having high cluster mean values for one or other character to be improved is likely to be more useful. The maximum contribution in manifestation of total genetic divergence were made by plant height (76%) followed by days to maturity (9%). The minimum contribution of days to 50% flowering (1%) and 100-seed weight (1%) toward genetic divergence. The role of number of pods/plant (2%), biological yield/plant (6%), harvest index (5%) were medium range in manifestation of total genetic divergence in the available germplasms.

Keywords: Crop improvement, genetic divergence, *Pisum sativum*, path coefficient analysis, non-hierarchical Euclidean cluster analysis

Introduction

Field pea (*Pisum sativum* L. var. *arvense*) is amongst the most important legume crop of India, belongs to family Leguminosae, largely confined to cooler temperate zone between the tropic of Cancer and Mediterranean region (Anonymous, 2001; Singh *et al.* 2011; Singh *et al.* 2018) [15]. Being an important protein source, there is increase in demand for this pulse crop both for animal feed as well as for human consumption (Santalla *et al.* 2001; Bhuvaneshwar *et al.* 2017) [13, 2]. Its improvement is mainly based on exploiting the natural sources of germplasm by means of selection or hybridization followed by selection (Zohary and Hopf, 2000; Georgieva *et al.* 2016) [22, 8]. Important genetic diversity collections of *Pisum* with over 2000 accessions are found in national genebanks in at least 15 countries, with many other smaller collections worldwide (Coyne *et al.* 2011; Smykal *et al.* 2011; Smýkal *et al.* 2012; Georgieva *et al.* 2016) [4, 18, 17, 8].

The prime objective of most of the plant breeding programmes is to make an improvement in yield, but yield is a complex character and a combined result of a number of component traits (Carter *et al.* 2015) [3]. Yield is controlled polygenitically and influenced greatly by the environmental fluctuations. Therefore, the selection of superior genotypes based on yield alone would not be effective. In order to make the selection effective one has to put attention on the component characters, which contribute yield in positive direction. In such a situation the available variability in the gene pool provides an opportunity for selecting superior genotypes, which can be obtained through vigorous screening and evaluation. The knowledge of association between the characters and their direct and indirect contribution towards expression of seed yield will be an additional help to plant breeders in deciding the selection criteria. The identification of donor parents for important characters, assessment of genetic variation and diversity in the available genotypes and the information about character associations are required for planning a successful breeding programme.

Keeping this in view, the present investigation was undertaken to work out the direct and indirect effects of characters on seed yield by path coefficient analysis and also study the genetic divergence in the germplasm using non-hierarchical Euclidean cluster analysis.

Materials and Methods

The experiment was conducted at Genetics & Plant Breeding Research Farm of Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad

(U.P.). The experimental material comprised of 120 field pea germplasm including 40 indigenous and 10 exotic collections of pea and 4 check varieties viz., HUDP-15, HFP-4, HUP-2, Rachna. The check varieties are the released and widely adopted dwarf and tall varieties grown in the region. Germplasm lines were procured from "AICRP on MULLaRP crops" project collections of pulse section of the department. These germplasm lines exhibited wide spectrum of variability and collected from IIPR, Kanpur and maintained in the department.

Layout plan

The experiment was conducted to evaluate one hundred twenty germplasm lines along with four checks under irrigated normal soil condition in Augmented Block Design. The entire experimental field was divided into 6 blocks of equal size and each block had twenty four entries including four checks. Consisted single row of 2.75 meter length, following inter and intra row spacing of 30 cm and 10 cm, respectively. Recommended cultural practices were applied to raise a good crop.

Observations Recorded

Five competitive plants from each plot were randomly selected for recording observations for all the quantitative characters except days to 50 per cent flowering and days to maturity, which were recorded on line basis. Average data from randomly selected plants of each block with respect to different characters were used for various statistical analyses.

(1) Days to 50 per cent flowering

The days to 50 per cent flowering were recorded from the date of sowing to date on which 50 per cent plants of each plot flower.

(2) Days to maturity

It was recorded as the number of days taken from the date of sowing to complete maturity i.e., plant became straw coloured and the pods were completely dry.

(3) Plant height (cm)

Plant height at maturity was measured on sampled plants in centimeters from the ground level to the top of the plant and averaged.

(4) Number of primary branches per plant

The number of primary branches per plant was counted from randomly selected plants when it attained complete vegetative growth.

(5) Number of pods per plant

Total number of pods per plant in each plant were counted at the time of maturity and averaged.

(6) Number of seeds per pod

Five pods were randomly taken from total pods of five randomly selected plants from each germplasm. Total number of seeds of these pods was counted and their mean value was expressed as the number of seeds per pod.

(7) 100-seed weight per plant (g)

The seeds of selected plants from each plot were mixed to draw a representative sample of 100-seeds which was weighed in grams.

(8) Biological yield per plant (g)

At maturity, the whole plant weight, including shoot and dried leaves, on randomly selected five plants was taken in grams and mean value was expressed as biological yield per plant (g).

(9) Harvest index (%)

It was computed as follows:
Harvest index (%) =

(10) Seed yield per plant (g)

After threshing and cleaning, the seeds obtained from each sampled plant were weighed in grams and mean value was taken as seed yield per plant.

Statistical Analysis

The experimental data collected in respect of 10 characters on 120 field pea genotype and 4 checks were compiled by taking the mean values of selected plants in each plot and subjected for following statistical analysis

1. Path coefficient analysis and
2. Non hierarchical Euclidean cluster analysis.

Path Coefficient Analysis

Path coefficient analysis was carried out according to Dewey and Lu (1959). Seed yield was assumed to be dependent variable (effect) which is influenced by all the nine characters and the independent variables (causes), directly as well as indirectly through other characters. The variation in seed yield unexplained by the nine causes was presumed to be contributed by a residual factor effect (x) which is uncorrelated with other factors. Path coefficients were estimated by solving the following simultaneous equation indicating the basic relationship between correlation and Path coefficient. The equations used are as follows:

$$r_{ij} = P_{iy} + \sum_{j=1}^{10} r_{ij} P_{ij} \text{ for } i=1,2,\dots,10$$

$$r_{ij} = \sum_{j=1}^{10} r_{ij} P_{ij} \text{ for } r_{ij} = 1$$

The above equation can be written in form of matrix.

$$[A]_{10 \times 1} = [B]_{10 \times 1} [C]_{10 \times 1}$$

Where,

A is column vector of correlation r_{ij} .

B is correlation matrix of r_{ij} , and

C is column vector of direct effect, P_{iy}

Residual factor was calculated as follows:

$$P_{xy} = \sqrt{1 - R^2}$$

Where,

$$R^2 = \sum_{j=1}^{10} P_{iy} r_{ij}$$

The r_{ij} i.e. $r_{1.2}$ to $r_{9.10}$ denote correlations between all possible combinations of independent characters P_{1y} to P_{10y} denote direct effect of various characters on character y .

r_{iy} = correlation coefficient between i^{th} and y characters

P_{iy} = Direct effect of i^{th} character on y .

Non- hierarchical Euclidean Cluster Analysis

Genetic divergence among 204 genotypes including checks planted in augmented design was studied through Non-hierarchical Euclidean cluster analysis (Beale, 1969 and Spark, 1973) [1, 19].

According to Beale (1969) [1], initially each observation is allocated to its closest cluster centers. The mean of the clusters are calculated and are taken to new cluster centers. At the same time, the sum of squared deviation of the observation from their respective cluster centers is computed. The observation are checked in turn to see if a shift to a different cluster centers results in a decrease in the total sum

of squares. This assumes that d_i^2 is less than d_k^2 , where d_i is the distance from the cluster centers i . However a more effective criterion involves reassigning the observation if the squared deviation from the center of cluster "i" is less than that from center of cluster k , even when the cluster centers are simultaneously repositioned. This is when:

$$\frac{ni}{ni+1}d_i^2 < \frac{nk}{nk-1}d_k^2$$

Where,

Where,

x_i is the means of x_i over the 'm' members.

$$\frac{1}{m(m-1)} \sum_{i=1}^p \left[\sum_j \sum_k (x_{ij}-x_i)^2 + \sum_j \sum_k (x_{ik}-x_i)^2 - 2 \sum_j \sum_k (x_{ij}-x_i)(x_{ik}-x_i) \right]$$

The cross product vanished and other two are equal.

$$\text{Thus average deviance} = \frac{2}{m-1} \sum_{i=1}^p \sum_{j=1}^m (x_{j}-\bar{x}_i)^2$$

Now instead of calculating $1/m(m-1)$ deviation, 'm' deviation from the centre of gravity is calculated.

The assumption in this method is the Euclidian distance 'D' separating 'n' points in a 'p'-dimensional space are proportional to the dissimilarities between the objects and secondly, that no object can belong simultaneously to two clusters.

Initially, a given number of vectors of cluster centers are located in the 'p' space. The position of these centers can be chosen arbitrarily or randomly. However, a good choice of initial cluster centers reduces the amount of computation to considerable extent.

To start with 'n' cases are allotted to a predetermined maximum number of clusters (C max) according to the procedure suggested by Beale.

The residual sum of squares RSS for the solution involving 'c' is reduced by 1 (unless $c = \text{min.}$) and this procedure is repeated till 'c' min. is recorded, i.e. further reduction is negligibly small. For each step RSS (c) is calculated when RSS (c) values for $c \text{ max.} \geq c \geq c \text{ minimum}$ are available, these are used in a pseudo-F-ratio test of the null hypothesis that the solution for the C_1 cluster provide no better fit than the solution for the C_2 clusters, with $C_1 > C_2$. This F-ratio is calculated as:

$$F = \frac{\frac{RSS(C_2) - RSS(C_1)}{C_2 - C_1}}{\frac{RSS(E_1)}{n - C_1 - C_2 - P - 1}}$$

With $P(C_2 - C_1)$ and $P(n - C_1)$ d.f. the null hypothesis is rejected if the calculated F exceeds the table value of F.

For reducing the number of clusters by 1 till C minimum is reached. Beale has suggested certain procedures. Instead of using Beale's procedure for merging two clusters, Doshi *et al.* (1990) [7] have adopted a simple procedure. When a solution is found for 'c' cluster 'c' vectors of new clusters are calculated. From this set of new cluster center vectors, last vector is dropped and (c-1) clusters. For determining the appropriate number of clusters, F test gives a rough guide in exploratory analysis

Results and Discussion

Path Coefficient Analysis

Path coefficient analyses were estimated on phenotypic as well as genotypic levels to resolve the direct and indirect effects of different characters on seed yield are presented in Table 1. At phenotypic level the highest positive direct effect on seed yield per plant was exerted by biological yield/plant (0.87) followed by harvest index (0.28). Whereas very low

n_i is the number of observations in cluster i .

In the limiting cluster usually average deviance among a subset of 'm' points is considered, not the individual $1/2 m(m-1)$ deviances. If the i^{th} variable on the j^{th} number is x_{ij} , average deviance of a set of m is as follows

$$\frac{1}{m(m-1)} \sum_{i=1}^p \sum_{j=1}^m \sum_{k=1}^m (x_{ij}-x_{ik})^2$$

$$\frac{1}{m(m-1)} \sum_{i=1}^p \sum_{j=1}^m \sum_{k=1}^m [(x_{ij}-x_i)(x_{ik}-x_i)]^2$$

positive and direct effect was recorded by 100-seed weight (0.01). Direct effect in negative direction was exerted by the days to 50 percent flowering (-0.01), days to maturity (-0.02) and number of seed per pod (-0.01). Other characters contributing substantial direct effect on seed yield were plant height (0.01), number of primary (0.02) and 100-seed weight (0.01). This indicated that direct contribution of these traits was too low to be considered of any consequences.

Highly positive indirect effects on seed yield per plant by harvest index (0.056), number of primary branches per plant (0.01) and plant height (0.00) via biological yield per plant; biological yield per plant (0.18), number of primary branches per plant (0.01), 100-seed weight (0.001) and plant height (0.001) via harvest index. However, biological yield per plant (-0.012) via 100-seed weight, biological yield per plant (-0.046) via days to 50 percent flowering, number of seed per pod (-0.002) via biological yield per plant showed highly negative indirect effects on seed yield per plant. The remaining estimates of the indirect effects in the analysis were too low to be considered. The estimate of residual effect (0.1842) and R^2 (0.9991) was negligible.

Genetic Divergence Analysis

The study of genetic divergence among the one hundred twenty four field pea genotypes was carried out by using Non-hierarchical Euclidean cluster analysis method was described by (Beale, 1969 and Spark, 1973) [1, 19].

The clustering patterns of one hundred twenty genotypes were grouped into twelve different non-overlapping clusters Table 2. Cluster X had highest number of genotypes (20) followed by cluster II and IX (14), cluster IV and XI (13) while, cluster XII and VII contained twelve and eleven genotypes, respectively. Lowest number of genotypes (4) recorded in III, V and VI.

The estimates of intra and inter cluster distances represented by D^2 value given in Table 3. The highest intra cluster distance observed in cluster V (16.706) followed by cluster VII (11.42), while, the lowest values recorded in case of cluster III (5.40). The maximum inter cluster distance recorded between cluster VI and cluster VIII (53.045) followed by cluster VI and cluster X (51.865) and cluster II and cluster VI (48.637), which suggested that members of these two clusters are genetically very diverse to each other. The inter cluster values between cluster XI and XII (12.910) and cluster X and XII (12.925) were also very low. The minimum inter cluster D^2 values recorded in case of cluster XI and XII (12.910). The high inter cluster distance indicated greater divergence between the genotypes of these cluster, while low inter cluster were not much genetically diverse from each other.

The results of path analysis obtained under present study are also in agreement with the results of Narshinghani *et al.*

(1979) ^[11], Gupta *et al.* (1986) ^[9] and Kumar *et al.* (1995) ^[10]

Table 1: Direct and indirect effects of ten characters on grain yield per plant in field pea

S. No.	Characters	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of pods/ plant	Number of primary branches/ plant	Number of seeds/ pod	100- Seed weight (g)	Biological yield/ plant (g)	Harvest index (%)	Seed yield/ plant (g)
1	Days to 50% flowering	-0.006	-0.004	0.000	0.000	-0.001	0.000	0.001	0.000	0.000	-0.076
2	Days to maturity	-0.012	-0.018	-0.001	-0.003	-0.003	-0.003	0.001	-0.001	-0.001	0.070
3	Plant height(cm)	0.000	0.001	0.012	0.003	0.004	0.000	-0.001	0.004	0.001	0.342
4	Number of pods/ plant	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.320
5	Number of primary branches/ plant	0.002	0.004	0.005	0.004	0.019	0.008	-0.001	0.006	0.007	0.375
6	Number of seeds/ pod	-0.001	-0.001	0.000	-0.002	-0.003	-0.007	0.000	-0.002	-0.002	0.255
7	100- seed weight	-0.001	-0.001	0.000	0.000	0.000	0.000	0.007	0.000	0.001	0.039
8	Biological yield/plant(g)	-0.046	0.072	0.305	0.290	0.261	0.189	-0.012	0.879	0.176	0.942
9	Harvest index(%)	-0.012	0.017	0.022	0.029	0.099	0.069	0.042	0.056	0.281	0.463

Residual factor = 0.1842, R² =0.9661

Bold figures indicate direct effects.

The cluster mean for ten characters are given in Table 4. Perusal of Table showed considerable differences between the clusters mean for different traits. The genotypes of cluster I had minimum mean value for plant height (49.67). The genotypes of cluster II characterized by high mean performance for days to 50% flowering (78.738). Cluster III showed maximum mean values for plant height (142.93) and number of seeds per pod (5.47). The genotypes of cluster IV characterized by high mean performance for plant height (117.83). The entries appeared in cluster V took maximum for days to maturity (122.958), 100-seed weight (25.22) and harvest index (52.632). Cluster VI had high mean value for number of pods per plant (23.309), number of primary branches per plant (3.321), biological yield per plant (36.582) and seed yield per plant (19.086). Cluster VIII had low mean value for days to maturity (105.226) and seed yield per plant (3.250). Cluster IX had characterized for low mean value to number of pods per plant (11.891) and 100-seed weight (16.101). Cluster X had low mean value for number of primary branches per plant (1.536), number of seed per pods (3.779), biological yield per plant (13.423) and harvest index (36.362).

The overall review of the result obtained by genetic diversity study in present investigation revealed that the crosses between the entries separated by the large inter-cluster distances and having high cluster mean values for one or other character to be improved is likely to be more useful. The results of Non-hierarchical Euclidian cluster analysis obtained under present study are also in agreement with the result of (Singh, 1987; Rao and Narsinghani, 1987, Singh, 1999 and Sureja and Sharma, 2001) ^[14, 12, 16 20].

The analysis of character contribution towards genetic divergence between one hundred twenty four genotypes of field pea is given in Table 5. The maximum contribution in manifestation of total genetic divergence were made by plant height (76%) followed by days to maturity (9%). The minimum contribution of days to 50% flowering (1%) and 100-seed weight (1%) toward genetic divergence. The role of number of pods/plant (2%), biological yield/plant (6%), harvest index (5%) were medium range in manifestation of total genetic divergence in the available germplasm.

Other characters showed moderate contribution towards total genetic divergence the similar results were reported by (Tikka and Asawa, 1981; Singh, 1987 and Dixit *et al.* 2002) ^[21, 14, 6].

Table 2: Clustering pattern of one hundred twenty field pea genotypes on the basis of Non-hierarchical Euclidean cluster analysis for ten characters

Cluster	Number of genotypes	Genotypes
I	8	FP-10-173,FP-10-186,FP-10-178,FP-10-132,FP-10-174,FP-10-181,FP-10-185,FP-10-167
II	14	NDP-2, IPFD-8-1, PP-25, SDP-408, FP-517, FP-10-168, FP-10-107, FP-9-613, HFP-716, HFP-715, HUDP-15, HFP-4, FP-9-617, EC-381866
III	4	FP-10-140, FP-10-143, FP-10-137, FP-10-142
IV	13	FP-10-122, FP-10-121, FP-10-132, NDP-408, HFP-529, EC-356166, EC-281864, HUP-2, Rachana, RFP-2009-I, VL-42, HFP-9426, Pant-P-42
V	4	FP-9-611, FP-10-135, FP-10-184, FP-537
VI	4	3-9-1, FP-10-136, FP-523, FP-9-618
VII	11	FP-10-127, EC-330833, JPF-9-11, FP-10-128, HFP-701, FP-572, PantP-137, Pant-P-138, JPBB-3, IM-9102, FP-513
VIII	7	KPMR-851, RFP-30, DDR-87, KPMR-815, DMR-64, DMR-7, IPF-820
IX	14	IC-356316, EC-384890, EC-386742, FP-453, DMR-7, IC-267182, EC-349478, EC-414419, IC-396802, VL-40, EC-507770, Pantnalogy, DDR-79, VL-45
X	20	FP-511, IPF-9-11, EC-356318, EC-334160, HFP-531, LMR-20, EC-324110, DDR-80, LFP-451, KPMR-871, NDP-1, HFP-99078, IC-361173, HFP-424, IC-209105, HFP-302, EC-412842, FP-536, LFP-449, IC-208369
XI	13	FP-10-164, FP-10-123, FP-10-133, FP-10-182, HUP-16, KPMR-814, DMR-63, DDR-88, FP-10-131, RFP-2009-4, LFP-471, KPDS-1, FP-10-182
XII	12	FP-529, HFP-621, HFP-530, IC-208370, Pant-P-136, VL-51, HFP-554, HFP-554, PP-142, SKND-4-9, PP-79, HFP-531

Table 3: Estimates of average intra- and inter-cluster distances for twelve clusters in field pea

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	8.33	16.45	16.09	15.78	27.73	32.71	23.45	28.50	21.49	27.21	14.52	21.12
II		10.98	24.56	15.70	30.45	48.63	28.06	21.40	17.44	18.78	20.54	18.03
III			5.40	13.74	25.87	30.85	23.37	26.89	25.83	31.40	17.72	26.37
IV				7.12	22.34	28.36	16.82	24.24	14.58	20.9	16.27	18.74
V					16.70	23.16	24.52	41.76	39.22	43.29	26.49	32.36
VI						10.90	18.79	53.04	42.29	51.86	25.46	38.84
VII							9.11	25.11	18.14	24.51	14.00	17.53
VIII								11.42	16.71	18.58	19.37	15.00
IX									9.13	14.17	17.35	14.76
X										8.87	20.25	12.92
XI											9.90	12.91
XII												8.98

Table 4: Clusters means for ten characters in field pea

Cluster	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of pods/plant	Number of primary branches/plant	Number of seeds/pod	100-seed weight (g)	Biological yield/plant (g)	Seed yield/plant (g)	Harvest index (%)
Cluster I	76.70	118.52	49.67	19.05	3.04	5.68	18.60	18.65	7.77	43.72
Cluster II	78.73	121.42	52.03	12.37	2.19	4.45	20.81	13.79	5.96	42.16
Cluster III	75.33	119.33	142.93	23.22	3.22	5.47	22.20	14.06	6.18	44.15
Cluster IV	75.60	122.51	117.83	17.82	2.26	4.41	18.48	20.37	8.65	43.06
Cluster V	77.83	122.95	105.82	16.82	2.58	4.84	25.92	29.68	15.29	52.63
Cluster VI	69.70	112.02	118.50	23.30	3.32	4.88	20.23	36.85	19.08	51.84
Cluster VII	68.26	106.47	115.58	14.08	2.64	4.63	19.84	27.67	12.51	45.22
Cluster VIII	71.22	105.22	95.03	9.50	2.58	4.14	21.82	6.04	3.25	48.30
Cluster IX	72.51	108.92	92.43	11.89	2.24	4.04	16.10	15.16	6.24	40.00
Cluster X	70.48	106.55	62.81	14.98	1.53	3.77	21.25	13.42	4.71	36.36
Cluster XI	68.96	109.98	71.15	17.78	2.85	4.91	20.46	17.93	8.99	47.59
Cluster XII	68.83	107.20	62.56	13.82	1.76	4.51	21.26	14.71	7.00	47.26

Table 5: Per cent contribution in 10 characters towards total genetic divergence

Characters	Contribution (%)
Days to 50% flowering	1
Days to maturity	9
Plant height (cm)	76
Number of primary branches per plant	0.0
Number of pods per plant	2
Number of seeds per pod	0.0
100-seed weight (g)	1
Biological yield per plant (g)	6
Harvest Index (%)	5
Seed yield per plant (g)	0.0

Conclusion

Path analysis identified, biological yield per plant and harvest index as important components having direct effects on seed yield. Number of pods per plant, number of seeds per plant, plant height, number of primary branches per plant substantial showed positive effects via harvest index. Thus, the characters identified above as important direct and indirect yield components merits, due consideration in formulating selection strategy in field pea for developing high yielding varieties. Non-hierarchical Euclidian cluster analysis grouped one hundred twenty four genotype into twelve distinct clusters this

indicated existence of genetic diversity in the germplasm collection. The twelve clusters in divergence analysis contained genotypes of heterogeneous origin, thereby indicating no parallelism between genetic and geographic diversity. Therefore, crosses between the genotypes of clusters separated with high inter cluster distances are likely to through desirable segregants. In this context, cluster VIII had very high inter-cluster distance from cluster VI followed by cluster X and VI and cluster VI and II. The different cluster means for ten characters and genotypes having distinctly different mean performance for various characters were separated into various clusters. The maximum contribution towards genetic diversity made by plant height followed by days to maturity.

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