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RESEARCH ARTICLE

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Morphological and chemical markers associated with *Acyrthosiphon pisum* tolerance in hybrid pea lines

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Abstract

Aim of study: Determining morphological and chemical markers in hybrid lines of winter forage pea (*Pisum sativum* subsp. *arvense* L.) associated with *Acyrthosiphon pisum* tolerance for breeding programs.

Area of study: The experimental field of the Institute of Forage Crops, Pleven, Bulgaria, during the 2016-2018 period.

Material and methods: Six hybrid lines of winter forage pea were studied for tolerance to *A. pisum*. The field trial was conducted using a long-plot design and a natural background of soil (leached chernozem) supplied with major nutrients. An entomological net for sweeping was used once a week for aphid number recording. Stem height and leaf number were recorded and chemical composition was determined at the flowering stage. The coefficient of variation concerning aphid density was calculated and the stability and adaptability of lines was evaluated.

Main results: Hybrid lines 6 and 12A were stable, widely adapted to the changing environmental conditions and the aphid density was statistically the lowest (31.1 and 36.8 individuals/m^2, respectively). A significant positive interaction was found between aphid density and plant height, leaf area, protein, and phosphorus content. Lines 6 and 12A had lower stems (74.7 and 82.5 cm), smaller leaf areas (571.13 and 657.39 cm^2/plant), lower protein and P contents, and these markers defined them as aphid tolerant.

Research highlights: Incorporation of plant markers of pea lines, less preferred by aphids, is an efficient tool for improving breeding programs for aphid resistance.

Additional keywords: aphid density dependent; morphological traits; chemical traits.

Abbreviations used: CF (crude fiber); CP (crude protein); CV (coefficient of variation).

Authors' contributions: This manuscript has one author who conceived and designed the study, performed the study, analyzed the data and wrote the manuscript.

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Introduction

Use of different markers for resistance as genetic material in development of new pea cultivars is one of the most effective methods for protection and control against *Acyrthosiphon pisum* Harris & M. (Hemiptera, Sternorrhyncha, Aphididae). The introduction of resistant pea cultivars would help the farmers to reduce losses due to pea aphid and would provide an environmentally safer option to aphid control. A wide range of traits such as chemical, physiological, morphological and life-history traits serve as protection traits against herbivores (Agrawal & Fishbein, 2006; Stenberg *et al.*, 2006; Agrawal, 2007; Carmona *et al.*, 2011).

Plant morphology can have a different impact on herbivorous insects including changes in abundance, fecundity, population density, etc. Many authors reported that the aphid population growth was strongly affected by plant surface area in different cultivars (Underwood & Rausher, 2000; Fitt *et al.*, 2002; Rhainds & Messing, 2005). Sandstrom & Pettersson (1994), Kemal (2002) and others found a reduction in fecundity and population growth rate of pea aphids on leafless or semi-leafless pea genotypes as compared to normal leaf genotypes. Higher aphid densities on larger leaves were found, suggesting that surface area could influence colonization and reproduction. The authors suggested that genotypes with a smaller leaf area provided less area for the aphids to colonize and feed.

Further it was mentioned that open canopies increased aphid exposure to adverse weather conditions and natural enemies.

Opposite effects of plant morphology on pea aphids were recorded. For example, Legrand & Barbosa (2000) reported that changes in leaf morphology of pea plant lines did not have a significant effect on pea aphid fecundity and population growth. Although the leaf area did not result in a fecundity reduction, longevity was significantly influenced both by leaf type and stipule size. Similarly, Soroka & MacKay (1991), in an earlier study, found no effect of morphology on population growth rates.

Considerable attention was paid to the influence of varietal differences of plants on aphid infestation in different studies. In searching the relative plant resistance to aphid impact, the host chemical composition possibly plays the most important role.

Nitrogen (N) is frequently considered as a limiting resource for *A. pisum* (Moravvej & Hatefi, 2008; Nikolova, 2017). Many authors have reported that the aphid density and fertility are often significantly lower in plants having lower nutritional qualities (Woods *et al.*, 2004; Moravvej & Hatefi, 2008; Vannette & Hunter, 2009). According to these authors, insects generally prefer higher concentrations of N and phosphorus (P) in leaves. However, Silva *et al.* (2005) detected negative correlations between *Acyrthosiphon* spp. density and N concentration, as well as a significantly higher concentration of N in an aphid resistant cultivar. On the other hand, Buchman & Cuddington (2009) found that pea aphids did not respond to changes in plant N or differences in K. Moreover, Babikova *et al.* (2014) found that the attractiveness of plants to pea aphids (*A. pisum*) was neither affected by P treatment nor correlated with leaf P concentration. Obviously, there are different viewpoints concerning the effect of morphological and chemical plant traits on the aphid response, which requires additional researches.

The decrease of pea aphid attacks by finding plant markers related to tolerance of winter forage pea genotypes and the development of varieties less preferred by aphids are of great importance for breeding programs. Therefore this study aimed at determining morphological and chemical markers in hybrid lines of winter forage pea associated with *A. pisum* tolerance.

Material and methods

During the 2016-2018 period in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria (43° 23.312' N; 24° 34.856' E; altitude 230 m), a study was conducted on the tolerance of six hybrid lines (number 6, 14, MR, 13, PL and 12A) of *P. sativum* subsp. *arvense* (winter forage pea) associated with *A. pisum*. The field trial was conducted using a long-plot design with a sowing rate of 120 germinating seeds/m^2 in three replications, a plot of 6 m^2 and a natural background of soil supplied with the major nutrients. In the long-plot design, the replications were arranged in an elongate strip, *i.e.* the replications were arranged one after the other. This method was applied because the soil fertility was equalized. The soil type was a leached chernozem with pH (KCl) 5.49; and content of: total N, 34.30 mg/1000 g soil; P_20_5, 3.72 mg/100 g soil; K_{20}, 37.50 mg/100 g soil. No pesticides were applied.

An entomological net for sweeping was used once a week from April to June for aphid number recording. The average number of aphids was calculated for these months.

At the flowering stage (May) stem height and leaf number were recorded per plant using 20 plants of each replication. All leaves were taken from the measured plant to determine the leaf area. Subsequently, the leaves were scanned and the leaf area was calculated using the open platform for scientific image analysis ImageJ. Pea lines have a normal pea leaf, which is compound, consisting of a leaf stalk (rachis), different pairs of leaflets followed by an unpaired number of tendrils.

The coefficient of variation (CV, %) concerning aphid density was calculated and a three-step scale of variation was used as follows: the CV values can be 0-10, low variation; 11-20, moderate variation; >20, high variation (Lyubishchev, 1986). The regression coefficient (*bi*) was computed according to Finlay & Wilkinson (1963) in order to evaluate the stability and adaptability of lines depending on the aphid attack.

Aboveground biomass samples were taken at the flowering stage to determine the chemical composition by standard methods of the Weende system (AOAC, 2001). They included crude protein (CP) by Kjeldahl method (calculated by the formula CP=total N × 6.25); crude fiber (CF); phosphorus content (P), colorimetrically by hydroquinone; and Ca content, complexometrically.

Data were processed using ANOVA for an one-factor case, the mean being compared by a Tukey test at 5% probability ($p \leq 0.05$). Relationships between aphid variable and certain plant traits and chemical composition were tested using linear quadratic regression models and a multiple regression analysis. The statistical processing of experimental data was conducted using the Statgraphics Plus software program and PBSTAT program.

Results

The weather conditions over the years had an impact on *A. pisum* development and reproduction. The months of March and April in 2016 were characterized by a higher average daily temperature and air humidity (by 0.6 and 0.8ºC and by 7 and 1% humidity compared to 2017 and 2018) as well as by a higher rainfall (by 66.1 and 31.4 mm) (Table 1). These conditions led to the early appearance of aphids compared to the other years. The plants were at the sensitive stage of flowering and pod formation to aphid infestation in May and the first ten days of June. The drier and cooler weather during the same period in 2016 (by 0.7 and 3.3ºC temperature and by 107.3 and 28.8% rainfall compared to 2017 and 2018) suppressed population growth rates of the pea aphid, and its number was lower than in 2017 and 2018.

The quantitative presence of *A. pisum* in winter pea lines during the vegetation period was expressed to varying degrees and determined the preference of the species (Table 2). In 2016, the average population density of the pea aphid, in most lines, varied narrowly and did not exceed the mean value for the year. A significantly lower number was found in hybrid line 6 in comparison with lines 14 and 12A ($F_{5,59}$=12.867; p=0.0384). Lines 13 and PL were distinguished for the significantly highest number of *A. pisum* exceeding the average annual value by 86.7 and 93.2%, respectively. The population density of aphids in 2017 was higher than in the previous year, with an increase of 24.3%. The lowest number of the pea aphid was recorded in lines 6 and 12A and the differences, as compared to the other lines, were statistically significant ($F_{5,59}$=11.646; p=0.0214). The significantly highest density was established in hybrid line 13 followed by PL, which were highly preferred by the species. The number exceeded the mean value by 148.6% and 11.3%, respectively. A similar trend of the aphid choices was outlined in the third year of the study (Table 2). The pronounced preference of the aphids for lines 13 and PL was retained and the density exceeded the average by 79.6 and 39.7%, respectively. The differences between the above-mentioned lines and the other lines were statistically significant ($F_{5,59}$=6.803; p<0.001). Lines 14 and MR occupied a middle position, while lines 6 and 12A had a significantly lower aphid density.

The classification of the CV, as a precision measure in the experiment, was used to determine a variation for the density variables of *A. pisum* (Table 2). In that relation, it characterized the stability rate of the studied indicator in these hybrid lines. The CVs over the years had low values in the range of 0-10%, determining a low density variation. That showed a stable response of pea aphid in the choice and preference for host plants. A moderate variation of 10 to 20% was observed only in line 13 in 2016 and MR in 2017. The average data for the period showed that lines 6 and 12A were the least preferred by *A. pisum* and the density was statistically the lowest ($F_{5,59}$=7.565; p<0.001). That choice was stable over the years, which allowed to define the lines as tolerant. Lines 14 and MR occupied an intermediate position. Line 13 was highly sensitive to aphid infestation with a significantly higher density followed by line PL. The low CV determined a stable position of strong preferences for those hybrid lines.

A stable genotype possesses an unchanged performance regardless of any variation of the environmental conditions. This concept of stability is useful for quality traits, disease, and insect pest resistance, or for stress characters (Alberts, 2014). The parameter used to describe line stability in regard to aphid choice was also a regression coefficient (*bi*).

The variance analyses of aphid density in this study are presented in Table 3. There were statistically significant differences (p<0.01) for environments, genotypes, and genotype × environment interaction which showed the participation of different genetic systems in its control.

According to Eberhart & Russell´s model (1966), regression coefficients approximating one indicate line stability. Regression coefficients (*bi*) ranged from -0.7 to 2.11 (Fig. 1). That large deviation in coefficients showed different line reactions to environmental changes. Parameter values determined lines 6 (*bi*=1.20) and 12A (*bi*=0.86) as stable hybrid lines. Line 6 exhibited good adaptability in any environment, whereas number 12A had good adaptability in unfavorable environments. These lines occupied stable positions with significantly

Table 1. Meteorological characteristics of the Pleven region.

Months	Temperature,°C			Humidity, %			Rainfall, mm		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
March	8.4	10.3	5.3	74	64	75	76.6	46.1	98.1
April	15.3	12.2	16.9	66	61	62	73.1	37.5	20.2
May	16.3	17.0	19.6	70	73	65	47.7	155	76.5
June	22.3	23.0	21.8	67	64	68	45.8	44.8	155.2

Table 2. *Acyrthosiphon pisum* density (average number of individuals/m^2) and coefficient of variation (CV, %) in winter pea hybrid lines.

Hybrid lines	Density[1]	SD	CV	Density
		2016		
6	16.0	a	8.3	4.0
14	37.7	b	9.6	8.8
MR	21.5	ab	3.3	5.2
13	90.2	c	6.2	17.4
PL	93.3	c	8.3	6.1
12A	30.8	b	3.3	7.3
Mean	48.3			8.1
		2017		
6	28.1	a	3.9	8.8
14	49.3	b	5.1	5.1
MR	52.9	b	7.6	14.4
13	158.6	d	11.1	7.0
PL	71.0	c	7.9	9.7
12A	30.0	a	6.5	7.4
Mean	63.8			8.7
		2018		
6	49.2	a	4.8	9.7
14	64.1	b	3.9	6.1
MR	65.4	b	2.4	3.6
13	108.5	d	3.5	3.2
PL	84.4	c	3.3	4.9
12A	44.5	a	4.5	10.0
Mean	69.4			**6.3**
		2016-2018		
6	31.1	a	2.7	8.9
14	50.4	b	3.1	6.1
MR	46.6	b	1.6	3.4
13	119.1	d	3.2	2.7
PL	82.9	c	6.8	8.2
12A	36.8	a	5.6	10.3
Mean	61.1			6.6

[1]Means in each column followed by the same letters are not significantly different (p<0.05).

lower aphid preference; therefore, the least risk of changing the *A. pisum* density is found under changing environmental conditions and meteorological factors. That implies that they are stable, widely adapted and valuable for the selection. Line 14 had a primarily unstable response in that respect (bi=1.40). Lines MR, PL and 13 (bi = 2.11, –0.70, and 1.82, respectively) occupied an unstable position, suggesting their high sensitivity to changing environmental conditions. They were distinguished for high numbers of aphids and are not recommended for selection purposes.

The hybrid lines differed in morphological traits, which had an effect on the aphid population abundance (Table 4). The stem height of the winter pea lines varied among them, the plants of line 13 being the highest, regardless of the specific agrometeorological conditions of the studied years (2016: $F_{5,59}$=9.610, p<0.001; 2017: $F_{5,59}$=14.023, p=0.044; 2018: $F_{5,59}$=11.153, p=0.038). Moreover, line 13 was identified with the highest plants on average for the period ($F_{5,59}$=5.151, p=0.026). Higher values of the marker were found in line PL with significant differences compared to the other lines in 2016 and on average for 2016-2018. Lines 6 and 12A had a stable position with significantly lower plants. An exception was observed with regard to lines 14 and MR in 2016 due to non-significant differences. Hybrid lines 14 and MR occupied a marked intermediate position because of similar height values.

The CVs were characterized by a low variation, which determined the marker stability. The interrelationship between aphid density and plant height was highly positive. The correlation coefficient was in the range of 0.887 ÷ 0.991, which expressed the aphid preference for higher plants.

The CV of leaf number varied in the range of 10-20% and a moderate variation was established. Over the years clear trends were not observed. A smaller number of leaves with significant differences, as compared to the other lines, were recorded in lines 6, 14 and PL in 2016 ($F_{5,59}$=23.998, p=0.039). The significantly highest values were found in line 13 in 2017, 2018 and on average for the period ($F_{5,59}$=30.637, p=0.040; $F_{5,59}$=13.796, p=0.0273; $F_{5,59}$=13.400, p=0.032, respectively), whereas the smallest leaf number was observed in line PL (no significant difference in line 14 both in 2018 and for the period). Line 14 had a smaller leaf mass, but there were no significant differences in hybrid plants of lines 6 and MR in 2017 and 2018. The correlation coefficient between the aphid density and leaf number varied from a slightly to moderately positive interaction (r=0.285 ÷ 0.454). The indicator had no obvious influence on the aphid preference.

Another variable reported in this study was the leaf area (Table 4) and clear pronounced differences between hybrid lines were outlined. Lines 6 and 12A were distinguished for the smallest area and significant differences compared to the other lines over the years and in 2016-2018 (2016: $F_{5,59}$=74.290, p<0.001; 2017: $F_{5,59}$=162.611, p=0.0218; 2018: $F_{5,59}$=272.393, p=0.046; 2016-2018: $F_{5,59}$=125.108, p<0.037). Hybrid line 12A had a significantly higher value than line 6 only in 2016. A larger leaf area was established in lines

Table 3. Analysis of variance for stability regarding to aphid numbers for the period 2016-2018.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value	p (p>F)
Environments	2	4093.084	2046.54**	1.1260	0.3844
REP (ENV)	6	10905.20	1817.53**	3.9723	0.0048
Genotypes	5	50897.94	10179.59**	9.8485	0.0013
G×E interactions	10	10336.18	1033.62**	2.2590	0.0414
PC1	6	7965.90	1327.65**	2.9000	0.0238
PC2	4	2370.284	592.57**	1.3000	0.2924
Residuals	30	13726.46	457.55	-	-

**p<0.05.

13 and PL both over the years and on average for the period, the marker values in line 13 being the highest in 2017 and for 2016-2018. Stable intermediate positions were found for lines 14 and MR.

The CVs had low values determining a low leaf area variation (Table 4). A pronounced preference of the pea aphids for the larger leaf area was found, because of a high positive correlation (r) ranging from 0.888 to 0.907. It was suggested that the larger leaf area of plants could be due to a higher number of leaves. However, we were unable to detect a similar interaction. The leaf area was reciprocal of the number of leaves and the correlation was moderate positive (r=0.353 ÷ 0.587).

Morphological markers related to lower stems and smaller leaf area changed the plant environment for pea aphid in such a way, which made the plant less attractive or exposed pea aphid to additional mortality factors (predators, parasites, extremes of temperature). From that viewpoint, lines 6 and 12A, defined as tolerant lines, created unfavorable conditions for the population growth of *A. pisum*.

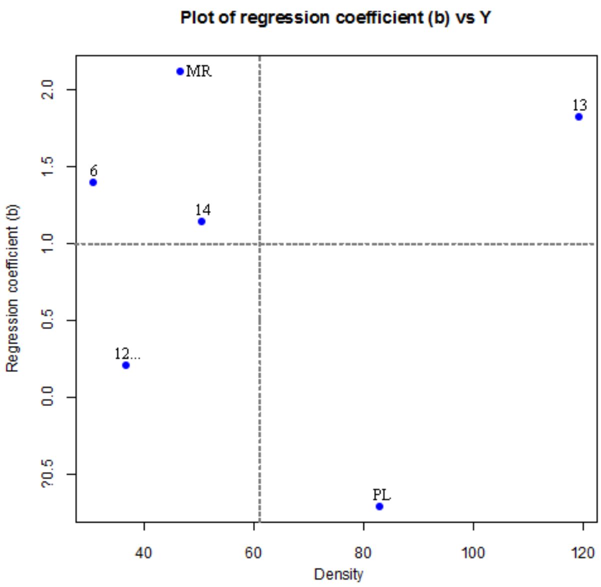

Figure 1. Stability and adaptability of pea hybrid lines according to regression coefficient (*bi*) and aphid density (average number of individuals/m^2).

The effect of plant traits such as height, leaf number and leaf area on *A. pisum* preference was examined by regression models (Fig. 2). A significant positive interaction was found between aphid density and plant height ($F_{1,17}$=53.68; p<0.001, R^2=0.238; Fig. 2A), as well as leaf area ($F_{1,17}$=37.33; p<0.001, R^2=0.457; Fig. 2C). Pea aphid density significantly increased with increasing plant height and leaf area in the lines studied. Pea aphid density was positively correlated with the leaf number too, but there was not a significant difference ($F_{1,17}$=4.12; p=0.069, R^2=0.221; Fig. 2B).

In a comparative analysis concerning the influence of the contents of chemical components in pea hybrid lines on their tolerance to *A. pisum* it was found that lines having lower CP and P content had a lower level of infestation (Table 5). For example, the tolerant lines 6 and 12A were characterized by the lowest CP content over the years, furthermore on average for the period, and the differences compared to the other lines were statistically significant (2016: $F_{5,17}$=11.530; p=0.001; 2017: $F_{5,17}$=5.463; p=0.005; 2018: $F_{5,17}$=5.543; p<0.001; 2016-2018: $F_{5,17}$=4.435; p<0.001). The preference of the pea aphid with reference to the CP, respectively N and P content, was related to a higher concentration. That resulted in a higher aphid abundance in hybrid lines 13 and PL. These lines had the highest protein content in the aboveground dry mass and the differences were significant in comparison with other variants (except for those between the lines MR and PL in 2016). The P content was significantly higher in the plants of lines 13 and PL (there was a non-significant difference between them and MR in 2017), while lines 6 and 12A had a significantly lower concentration (2016:

Table 4. Morphological characteristics of winter pea hybrid lines.

Hybrid lines	Height (cm)	CV	No. of leaves/ plant	CV	Leaf area (cm^2/ plant)	CV
2016						
6	98.3 b	9.5	64.3 a	16.8	522.90 a	10.3
14	93.6 ab	10.6	56.4 a	12.2	610.25 c	8.6
MR	89.5 ab	7.8	114.7 b	11.8	587.90 c	7.0
13	169.4 d	12.0	120.4 b	11.7	712.80 d	9.7
PL	153.5 c	9.1	76.5 a	8.6	648.36 d	6.8
12A	87.4 a	8.6	117.5 b	15.0	558.80 b	8.1
Mean	115.3		91.6		606.84	
2017						
6	59.6 a	7.4	99.8 bc	16.8	514.80 a	8.8
14	79.6 b	6.7	94.2 b	16.3	628.00 b	7.9
MR	74.7 b	10.5	109.8 bc	17.1	639.24 c	9.6
13	93.7 c	10.1	163.8 d	14.3	860.70 e	10.4
PL	79.5 b	6.8	61.5 a	13.4	663.60 d	6.3
12A	60.5 a	9.4	128.5 c	10.2	605.00 a	5.5
Mean	74.6		109.6		651.89	
2018						
6	66.4 a	6.3	85.6 c	11.7	675.70 a	4.6
14	98.9 c	8.4	66.6 ab	8.1	847.56 b	7.5
MR	101.6 c	10.6	77.4 bc	5.8	877.70 b	8.7
13	122.4 d	9.4	192.2 e	9.9	2295.20 c	6.7
PL	106.9 c	8.9	60.2 a	6.2	2209.00 c	10.8
12A	84.1 b	8.2	141.6 d	10.0	808.36 a	9.8
Mean	96.7		103.9		1285.59	
2016-2018						
6	74.7 a	4.5	83.2 b	9.0	571.13 a	8.9
14	90.7 b	9.8	72.4 a	11.8	695.27 b	5.1
MR	88.6 b	8.7	100.6 b	7.1	701.61 b	7.3
13	128.5 d	10.7	158.8 d	6.2	1289.57 d	5.4
PL	113.3 c	6.3	66.1 a	8.9	1173.65 c	9.3
12A	82.5 a	5.4	129.2 c	12.3	657.39 a	9.7
Mean	97.7		104.0		848.10	

CV: coefficient of variation, %. Means in each column followed by the same letters are not significantly different (p<0.05).

$F_{5,17}$=0.031; p=0.002; 2017: $F_{5,17}$=0.028; p=0.001; 2018: $F_{5,17}$=0.027; p<0.001; 2016-2018: $F_{5,17}$=0.024; p<0.001). The average results showed that lines 13 and PL were distinguished for significantly higher CP content by 11.6% and 12.0%, and P concentration by 27.1 and 14.4%, respectively, in relation to the mean values. On the other hand, lines 6 and 12 had lower CP content by 17.4 and 10.9%, and P concentration by 22.1 and 17.5%, respectively.

Regarding to the CF and Ca content, there was an opposite trend (Table 5). The lines characterized by higher concentrations were relatively less frequented and preferred by *A. pisum*. The dependence was more pronounced with regard to the Ca concentration. The slightly infected lines 6 and 12A had a significantly higher Ca proportion than the other lines in 2017, 2018 and on average for 2016-2018, exceeding the mean value by 4.6% and 14.6% for the studied period (2017: $F_{5,17}$=0.024; p<0.001; 2018: $F_{5,17}$=0.080; p=0.003; 2016-2018: $F_{5,17}$=0.026; p=0.002). In the first year, the differences between the hybrid lines were mostly nonsignificant (2016: $F_{5,17}$=0.088; p<0.0341). A similar trend of significantly higher CF content in the plants less preferred by aphids was established in lines 6 and 12 A in 2018 and on average for the period (2018: $F_{5,17}$=12.499; p=0.001; 2016-2018: $F_{5,17}$=11.029; p=0.010). In the first two years the lines took the middle positions concerning the CF content (2016: $F_{5,17}$=7.715; p<0.001; 2017: $F_{5,17}$=12.499; p=0.001).

Regression models for the effects of chemical components on the *A. pisum* density were presented in Fig. 3. A significant positive interaction between aphid density and protein content ($F_{1,17}$=17.75; p=0.001; R^2=0.484; Fig. 3A), as well as the P content ($F_{1,17}$=45.98; p=0.000; R^2=0.214; Fig. 3D) was detected. The hybrid lines containing a lower concentration of P and CP were not attractive to the species diet, whereas the high content of these components was a reliable marker for aphid outbreak. On the other hand, there was a negative interaction between pea density and CF and Ca concentration. The higher Ca content was related to a significantly lower density ($F_{1,17}$=2.26; p=0.015; R^2=0.317; Fig. 3C), while the CF effect was nonsignificant ($F_{1,17}$=45.98; p=0.000; R^2=0.214; Fig. 3B). Moreover, a high positive linear correlation was found between CP and P content and aphid density (average r=0.789 and 0.934, respectively). On the other hand, a weak and low negative correlation was expressed concerning Ca and CF contents, respectively (r= –0.035 and –0.395).

Morphological traits and chemical compounds play a primary role in host plant resistance to insect attack. Therefore, it is necessary to examine not only the individual effect of plant traits, but also their mutual impact on the aphid response. The applied regression analysis (ANOVA) in Table 6 shows that the interaction of plant traits had a significant effect on the population density of *A. pisum*. The P content had the highest regression coefficient (r=438.392) and strongly determined the choice of aphids (Table 6, below). It had a significant positive effect. The lines having a high concentration were highly preferred and probably provided favorable conditions for aphid

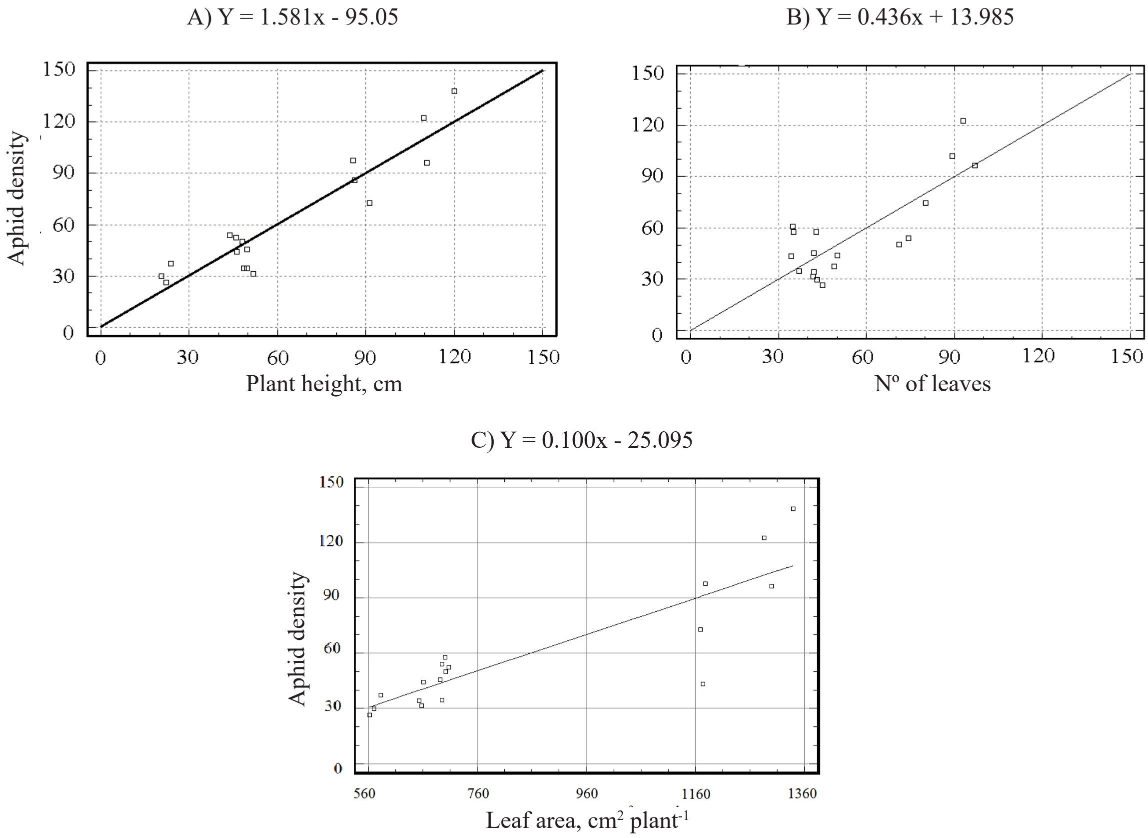

Figure 2. Effect of the morphological traits on the pea aphid density (average number of individuals/m^2).

diet and population growth rates. The leaf area had a significantly stronger positive effect on the numbers (*r*=2.967) in comparison with plant height (*r*=1.468) and CP content (*r*=1.035). The leaf number per plant had a nonsignificant effect. Crude fiber and Ca contents had a low and nonsignificant negative effect in the complex interaction between plant traits and aphid density.

Discussion

Factors such as plant morphological characteristics, nutrients, and others could change the size of insect populations (Agrawal, 2004; Rhainds & Messing, 2005). Stem and leaf dimension, branching angles, surface complexity are all-important morphological markers of a plant, which could influence insect pests. The results of the present study are consistent with other reports of positive effects of morphological markers on the population density of *A. pisum*. For example, Spahkov (2004) reported the effect of plant size of pea varieties on the aphid population. The author found that the higher plants were highly preferred by aphids than the lower ones. Furthermore, the pea aphid infestation was significantly positively correlated with the plant height. Geteneh (2018) found that the resistant lentil genotype had a significantly smaller leaf area and leaf number than the susceptible control. Moreover, the tolerance in a resistant genotype was expressed in a decreased percent of plant height and dry weight reduction. Similarly, Onyishi *et al.* (2013) studied different degrees of genetic diversity in terms of traits such as leaf area, seed number per pod, pod length, and variation in the infestation of insect pests, including aphids, among cowpea genotypes. Authors reported that the susceptible genotypes to aphid infestation had a considerably larger leaf area.

On the other hand, Fikru *et al.* (1999) hypothesized that plant physiological response, particularly photosynthesis and leaf area, substantially contributed to the plant tolerance to aphid injury. According to the authors, gradual photosynthetic compensation, larger leaf area, and more dry matter in tolerant lines could significantly contribute to plant tolerance resulting from aphid damage. In a later study, it was found that the aphid number per plant increased with the increase of total leaf area (Moravvej & Hatefi, 2008). Authors reported a positive significant correlation. Moreover, significant relationship was detected between plant height and aphid numbers. Similarly, Makasheva (1973) & Posylaeva (1990) previously reported for

Table 5. Chemical composition of pea dry mass of hybrid lines, g/kg dry matter.

Hybrid lines	Crude protein	Crude fiber	Ca	P
2016				
6	186.7 a	230.5 c	0.881 ab	0.294 a
14	225.9 c	225.5 c	0.859 ab	0.376 c
MR	239.5 d	210.1 b	0.854 ab	0.344 b
13	254.2 e	202.1 a	0.810 a	0.440 d
PL	230.5 cd	248.5 d	0.863 ab	0.417 d
12A	200.3 b	227.8 c	0.934 b	0.303 a
Mean	222.8	224.1	0.867	0.362
2017				
6	126.0 a	286.3 c	0.976 d	0.254 a
14	128.6 b	214.4 a	0.917 ab	0.296 b
MR	137.7 b	258.6 b	0.913 a	0.321 bc
13	134.5 c	263.5 b	0.940 bc	0.319 bc
PL	117.0 c	304.1 d	0.946 c	0.344 c
12A	126.0 a	268.3 b	1.022 e	0.264 a
Mean	128.7	265.9	0.952	0.300
2018				
6	107.9 a	273.3 c	1.610 d	0.149 a
14	143.7 c	245.7 b	1.438 b	0.229 b
MR	161.8 d	221.8 a	1.300 a	0.212 b
13	167.6 e	265.3 c	1.521 c	0.377 d
PL	196.35 f	252.1 b	1.270 a	0.261 c
12A	129.4 b	287.5 d	1.836 e	0.172 a
Mean	151.1	257.6	1.496	0.233
2016-2018				
6	138.1 a	263.4 c	1.156 c	0.232 a
14	165.2 c	228.5 a	1.071 b	0.300 b
MR	176.6 d	230.2 a	1.022 a	0.292 b
13	186.5 e	243.6 b	1.090 b	0.379 d
PL	187.1 e	268.2 d	1.026 a	0.341 c
12A	148.9 b	261.2 c	1.264 d	0.246 a
Mean	167.1	249.2	1.105	0.298

Means in each column followed by the same letters are not significantly different ($p<0.05$).

increased resistance to pea aphids in varieties having longer internodes, fewer flowers, and less leaf area. Such an effect was described by Kareiva & Sahakain (1990) where the plants of semi-leafless varieties and having a small leaf area strongly suppressed the *A. pisum* population. These varieties had resistance to pea aphid, which was considerably greater than the true inherent resistance of only about 8%.

Similar interactions were found in the present study. First, a stable response of pea aphid was found concerning the choice and population preference in the hybrid lines allowing to identify the factors responsible for the choice. The lines differed in morphological traits, which had a significant effect on the aphid density, and as a result, the lines having a smaller leaf area and height were less preferred by aphids. In addition, there was a significant positive effect between aphid density and plant traits.

Morphological markers have not been the only factor influencing the behavior of the pea aphid. The quality of food in terms of composition of different chemical compounds (*e.g.* N, Ca, P) was also important for the aphid infestation. According to Terra (1988), obtaining nitrogenous compounds is the main nutritional challenge facing aphids.

A large number of authors concluded that the high N inputs were an important factor contributing to a high aphid population and aphid fecundity (Cisneros & Godfrey, 2001; Ricklefs, 2008; Staley *et al.*, 2011; Gash, 2012; Santiago *et al.*, 2012). Vannette & Hunter (2009), and Bala *et al.* (2018) specified that the increased N and P content in plant tissues had positive effects on population growth and other parameters of aphid performance. Similar results were reported in a previous study where the high content of these components resulted in significantly greater *A. pisum* population density in winter vetch varieties (Nikolova, 2017). Godfrey *et al.* (2000) reported that applying high rates of N to cotton plants increased cotton aphid reproductive rates and could create conditions favorable to aphid outbreaks. According to Ricklefs (2008), the P content is one of the key limiting nutrients for an extensive range of insect pest. Furthermore, Münzbergová & Skuhrovec (2013) found that the leaf quality expressed as content of P, water, and specific leaf area had a more important effect on the insect damage than the presence of specific defense mechanisms such as spines and hair. Dadd (1973) reported that the insects needed considerable amounts of K, P, and Mg in their diets, whereas the required Ca, sodium and chloride amounts were small. In this direction, Moravvej & Hatefi (2008) noted that a higher number of pea aphid was observed in pea varieties when the soluble N concentration in the leaves was higher. Similar results are reported in the present study, where the preference of pea aphid was related to lines characterized by significantly higher CP and P content, and lower Ca concentrations (lines 13 and PL). Furthermore, these chemical markers, as well as the morphological ones, exhibited a significant positive effect in the mutual impact on pea aphid density. As a result, the P content had the highest effect followed by the leaf area, plant height, and CP.

In general, the plant traits such as height, leaf area, CP, and P concentration may be used as markers for pea aphid tolerance in hybrid lines of winter forage pea. The

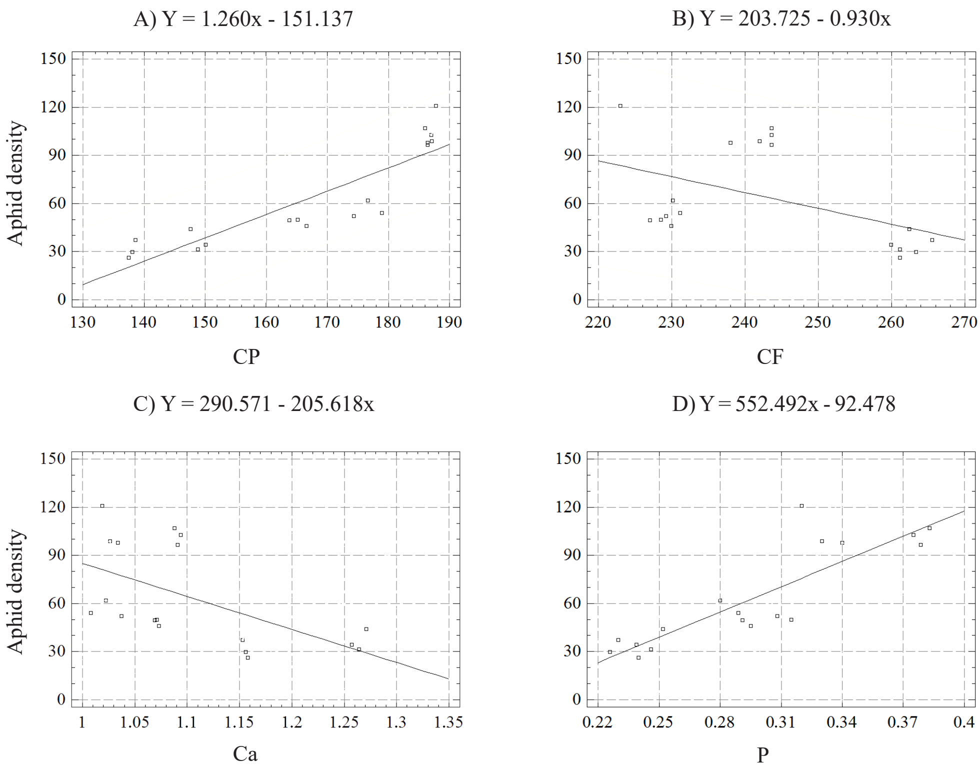

Figure 3. Effect of the chemical components on the pea aphid density (average number of individuals/m^2).

Table 6. Regression analysis (ANOVA) and regression coefficients of the pea aphid density in regard to the plant traits.

ANOVA	df	SS	MS	F	Significance F	
Regression	7	16906.500	2415.210	17.670	0.0001	
Residual	10	1366.550	136.655			
Total	17	18273.000				
Regression coefficients						
Factors	**Coefficients**	**SE**	**t Stat**	***p*-value**	**Lower 95%**	**Upper 95%**
Intercept	33.611	180.343	0.186	0.000	-368.220	435.442
Height	1.468	0.235	6.258	0.001	0.965	1.972
Leaf area	2.967	0.048	1.234	0.037	2.726	3.207
Number of leaves	0.409	0.191	1.960	0.070	0.035	0.784
Crude protein	1.035	0.561	1.808	0.044	-0.197	2.224
Crude fiber	-0.411	0.302	-1.364	0.196	-1.063	0.240
Calcium	-0.876	7.304	-0.847	0.412	-83.229	81.478
Phosphorus	438.392	168.496	2.602	0.022	74.378	802.405

SS: sum of squares. MS: mean squares. SE: standard errors.

present data showed that two pea hybrid lines (6 and 12A), which were distinguished for significantly lower stems and smaller leaf area, as well as for significantly lower CP and P content, were tolerant to *A. pisum* outbreak. The incorporation of plant markers for the selected pea lines is an efficient tool to be applied as marker-assisted selection, which greatly contributes to improving breeding programs for aphid resistance.

References

Agrawal A, 2004. Plant defense and density dependence in the population growth of herbivores. Am Natur 164: 113-120. https://doi.org/10.1086/420980

Agrawal A, 2007. Macroevolution of plant defense strategies. Trends Ecol Evol 22: 103-109. https://doi.org/10.1016/j.tree.2006.10.012

Agrawal A, Fishbein M, 2006. Plant defense syndromes. Ecology 87: 132-149. https://doi.org/10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2

Alberts MJA, 2014. A comparison of statistical methods to describe genotype x environment interaction and yield stability in multi-location maize trials. Master's thesis. Univ. free state Bloemfontein, South Africa, 100 pp.

AOAC, 2001. Official methods of analysis, 18th ed. Assoc Anal Chem, Gaithersburg, MD, USA.

Babikova Z, Gilbert L, Randall KC, Bruce TJA, Pickett JA, Johnson D, 2014. Increasing phosphorus supply is not the mechanism by which arbuscular mycorrhiza increase attractiveness of bean (Vicia faba) to aphids. J Exp Bot 65 (18): 5231-5241. https://doi.org/10.1093/jxb/eru283

Bala K, Sood AK, Pathania VS, Thakur S, 2018. Effect of plant nutrition in insect pest management: A review. J Pharmacogn Phytochem 7 (4): 2737-2742.

Buchman N, Cuddington K, 2009. Influences of pea morphology and Interacting factors on pea aphid (Homoptera: Aphididae) Reproduction. Environ Entomol 38 (4): 962-970. https://doi.org/10.1603/022.038.0402

Carmona D, Lajeunesse M, Johnson M, 2011. Plant traits that predict resistance to herbivores. Funct Ecol 25: 358-367. https://doi.org/10.1111/j.1365-2435.2010.01794.x

Cisneros JJ, Godfrey LD, 2001. Midseason pest status of the cotton aphid (Homoptera: Aphididae) in California cotton: is nitrogen a key factor? Environ Entomol 30: 501-510. https://doi.org/10.1603/0046-225X-30.3.501

Dadd RH, 1973. Insect nutrition: current developments and metabolic implications. Annu Rev Entomol 18: 381-420. https://doi.org/10.1146/annurev.en.18.010173.002121

Eberhart SA, Russel WA, 1966. Stability parameters for comparing varieties. Crop Sci 6: 36-40. https://doi.org/10.2135/cropsci1966.0011183X000600010011x

Fikru JH, Higley LG, Ni X, Quisenberry SS, 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. Environ Entomol 28 (5, 1): 787-794. https://doi.org/10.1093/ee/28.5.787

Finlay KW, Wilkinson GN, 1963. The analysis of adaptation in plant-hybrid programme. Aust J Agric Res 14: 742-754.

Fitt G, Mares C, Constable G, 2002. Enhancing host plant resistance of Australian cotton varieties. The Australian Cottongrower 23 (1): 20-26.

Gash AFJ, 2012. Wheat nitrogen fertilisation effects on the performance of the cereal aphid Metopolophium dirhodum. Agronomy 2: 1-13. https://doi.org/10.3390/agronomy2010001

Geteneh M, 2018. Resistance mechanism(s) in lentil genotype to pea aphid (Acyrthosiphon pisum Harris) (Hemiptera: Aphididae). Master's thesis, Haramaya University, Haramay, 152 pp.

Godfrey L, Rosenheim J, Goodell P, 2000. Cotton aphid emerges as major pest in SJV cotton. Calif Agr 54 (6): 26-29.

Kareiva P, Sahakain R, 1990. Tritrophic effects of a sample architectural mulation in pea. Nature 345 (6274): 433-434. https://doi.org/10.1038/345433a0

Kemal AA, 2002. An integrated approach to pest management in field pea, Pisum sativum (L.), with emphasis on pea aphid, Acyrthosiphon pisum (Harris). Master's thesis, Univ of the Free State, Ethiopian Agric Res Organ, 161 pp.

Legrand A, Barbosa P, 2000. Pea aphid (Homoptera: Aphididae) fecundity, rate of increase, and within-plant distribution unaffected by plant morphology. Environ Entomol 29: 987-993. https://doi.org/10.1603/0046-225X-29.5.987

Lyubishchev A, 1986. Dispersion analysis in biology. Moscow Univ Press, 200 pp.

Makasheva RK, 1973. Pea. Kolos, Leningrad, USSR.

Moravvej GH, Hatefi S, 2008. Role of nitrogen content of pea (Pisum sativum L.) on pea aphid (*Acyrthosiphon pisum* Harris) establishment. Caspian J Env Sci 6 (2): 113-131.

Münzbergová Z, Skuhrovec J, 2013. Effect of habitat conditions and plant traits on leaf damage in the Carduoideae subfamily. PLoS ONE 8 (5): e64639. https://doi.org/10.1371/journal.pone.0064639

Nikolova I, 2017. Factors affecting on the susceptibility of vetch cultivars to infestation by Acyrthosiphon pisum L. (Hemiptera, Aphididae). Russ J Ecol 48 (5): 482-490. https://doi.org/10.1134/S1067413617050095

Onyishi GC, Harriman JC, Ngwuta AA, Okporie EO, Chukwu SC, 2013. Efficacy of some cowpea genotypes against major insect pests in southeastern agro-ecology of Nigeria. Middle-East J Sci Res 15 (1): 114-121.

Posylaeva GA, 1990. A study on the pea aphid population structure [Исследование структуры населения гороховой тли]. Zashchita Rastenii (Kiev) 37: 42-45 [in Russian].

Rhainds M, Messing RH, 2005. Spatial and temporal density dependence in a population of melon aphids, Aphis gossyppii Glover (Homoptera: Aphididae), on established and sentinel taro plants. Appl Entomol Zool 40: 273-282. https://doi.org/10.1303/aez.2005.273

Ricklefs R, 2008. Foliage chemistry and the distribution of Lepidoptera larvae on broad-leaved trees in southern Ontario. Oecologia 157: 53-67. https://doi.org/10.1007/s00442-008-1050-x

Sandstrom J, Petterson J, 1994. Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (Acyrthosiphon pisum) performance. J Insect Physiol 40: 947-955. https://doi.org/10.1016/0022-1910(94)90133-3

Santiago L, Wright S, Harms K, Yavitt J, Korine C *et al.*, 2012. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. J Ecol 100: 309-316. https://doi.org/10.1111/j.1365-2745.2011.01904.x

Silva AA, Varanda EM, Primavesi AC, 2005. Effect of the inherent variation in the mineral concentration of alfalfa cultivars on aphid populations. Bragantia (Campinas) 64 (2): 233-239. https://doi.org/10.1590/S0006-87052005000200010

Soroka JJ, MacKay PA, 1991. Antibiosis and antixenosis to pea aphids (Homoptera: Aphididae) in cultivars of field peas. J Econ Entomol 84: 1951-1956. https://doi.org/10.1093/jee/84.6.1951

Spahkov S, 2004. Pests of soybean and forage pea in the conditions of the Voronezh forest-steppe area and methods of limiting their density. Master's thesis, Voronezh, Russia, 145 pp.

Staley JT, Stafford DB, Green ER, Leather SR, Rossiter JT, Poppy GM, Wright DJ, 2011. Plant nutrient supply determines competition between phytophagous insects. Proc Biol Sci 278: 718-724. https://doi.org/10.1098/rspb.2010.1593

Stenberg J, Witzell J, Ericson L, 2006. Tall herb herbivory resistance reflects historic exposure to leaf beetles in a boreal archipelago age-gradient. Oecologia 148: 414-425.

Terra W, 1988. Physiology and biochemistry of insect digestion: an evolutionary perspective. Brazil J Med Biol Res 21: 675-734.

Underwood N, Rausher MD, 2000. The effects of host-plant genotype on herbivore population dynamics. Ecology 81: 1565-1576.

Vannette RL, Hunter MD, 2009. Mycorrhizal fungi as mediators of defence against insect pests in agricultural systems. Agric For Entomol 11: 351-358.

Woods HA, Fagan WF, Elser JJ, Harrison JF, 2004. Allometric and phylogenetic variation in insect phosphorus content. Funct Ecol 18: 103-109. https://doi.org/10.1111/j.1365-2435.2004.00823.x