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Genetic analysis of photosynthesis-related traits in faba bean (*Vicia faba* L.) for crop improvement

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Abstract

Increasing productivity through improvement of photosynthesis in faba bean breeding programs requires understanding of the genetic control of photosynthesis-related traits. Hence, we investigated the gene action of leaf area, gas exchange traits, canopy temperature, chlorophyll content, chlorophyll fluorescence parameters and biomass. We chose inbred lines derived from cultivars Aurora (Sweden) and Mélodie (France) along with an Andean accession, ILB 938, crossed them (Aurora/2 × Mélodie/2, ILB 938/2 × Aurora/2 and Mélodie/2 × ILB 938/2), and prepared the six standard generations for quantitative analysis (P₁, P₂, F₁, F₂, B₁, and B₂). Gene action was complex for each trait, involving additive and dominance gene actions and interactions. Additive gene action was important for SPAD, photosynthetic rate, stomatal conductance and F_v/F_m. Dominance effect was important for biomass production. It is suggested that breeders selecting for productivity can maximise genetic gain by selecting early generations for canopy temperature, SPAD and F_v/F_m, then later generations for biomass. The information on genetics of various contributing traits of photosynthesis will assist plant breeders in choosing an appropriate breeding strategy for enhancing productivity in faba bean.

KEYWORDS *Vicia faba*, generation mean analysis, gas exchange, SPAD, chlorophyll fluorescence, canopy temperature

1 INTRODUCTION

Faba bean (*Vicia faba* L.) is a protein-rich and nutritious cool season grain legume crop that is widely grown for food and feed. As a legume with excellent ability to fix nitrogen, it provides ecosystem services that contribute to sustainable agriculture. Genetic approaches to improvement of faba bean productivity have been challenging because of the mixed breeding system of the crop (out-crossing range from 4-84 %, Bond & Poulsen, 1983). A recent study indicated that yield instability in faba bean has increased over time in Europe (Reckling et al., 2018). This may be attributable to the relative susceptibility of faba bean to environmental stresses such as high temperature, drought, and waterlogging (reviewed in Stoddard et al., 2006) and to biotic stresses (reviewed in Torres et al., 2006). Photosynthesis and its related traits are considered key determinants of genetic gains in yield potential and response to environmental stresses in crops (Evans, 2013; Mathan et al., 2016).

Genetic modification of stomatal function characteristics such as stomatal conductance has the potential to improve crop production through photosynthesis. These characteristics affect the uptake of atmospheric carbon dioxide into the leaf for photosynthesis and the loss of water through transpiration. Gas exchange characteristics are essential for enhanced yield potential in crop species (Roche, 2015). For example, high stomatal conductance in modern wheat (*Triticum aestivum* L.) varieties was correlated with cooler canopy temperature, higher photosynthetic rate and consequently yield (Fischer et al., 1998; Biswas et al., 2008). Moreover, in soybean [*Glycine max* (L.) Merr.], advanced cultivars were more efficient at producing and allocating carbon resources to seeds than were their predecessors due to increased photosynthesis and stomatal conductance along with decreased leaf area index (Morrison et al., 1999). Genotypic variation for gas exchange and morpho-physiological traits related to photosynthesis have been reported in faba bean (Khan et al., 2007; Khazaei et al., 2013; Alghamdi et al., 2015), suggesting that these characteristics may be useful for improving the adaptation of faba bean cultivars through breeding for specific production environments.

Many important traits in agriculture (e.g., yield, quality traits and some forms of disease resistance) are multigenic and quantitative. A study using molecular markers in chickpea (*Cicer arietinum* L.) indicated that 11 major QTLs (quantitative trait loci) were associated with photosynthetic efficiency parameters (e.g., chlorophyll content and gas exchange traits; Basu et al., 2019). Another study on the same species by Rehman et al. (2011) reported three QTLs for stomatal conductance and six QTLs for canopy temperature. In faba bean as well, stomatal conductance and canopy temperature had quantitative inheritance (Khazaei et al., 2014). Multiple gene control of photosynthesis-related traits has been reported in crop species (reviewed in Graham et al., 2008; Nishant et al., 2016). In plant breeding, the effectiveness of selection for a quantitative trait is primarily determined by the genetic effects driving its inheritance. The contributing genetic effects are additive, dominance and epistasis (additive × additive, additive × dominance, and dominance × dominance). Generally, additive effects are the average effects of the genes from both parental lines, while dominance and epistasis effects are the interaction of allelic and non-allelic genes affecting a trait, respectively. Single cross generation mean analysis (Mather &

Jinks, 1982) and diallel crosses (Gilbert, 1958; Falconer & MacKey, 1996) have been widely employed to study the genetic control of quantitative traits. Understanding the size and the nature of gene action and inheritance patterns helps breeding programs by assisting in the design of appropriate breeding methodology, resource management and selection procedures (effectiveness of early or late generation selection for specific traits).

Information on the genetic analysis required for improvement of complex physiological traits is limited, particularly gene effects through generation mean analysis of morpho-physiological traits related to photosynthesis in crop species. There are no reported estimates for gene action of gas exchange and photochemical activity traits in faba bean. Thus, the main objective of this study was to understand the nature and size of genetic control of photosynthesis-related traits in three faba bean crosses developed by crossing parental lines with contrasting photosynthetic characteristics and genetic backgrounds.

2 MATERIALS AND METHODS

2.1 Genetic material

The plant materials consisted of three faba bean inbred lines differing in photosynthetic activity, water use efficiency, drought response and productivity. The parent cultivar of Aurora was drought-susceptible with high productivity under non-drought conditions (Link et al., 1999) and came from Sweden. ILB 938 is an accession with high water use efficiency (WUE), low photosynthetic activity and low productivity (Khazaei et al., 2014) originating from the Andean regions of Ecuador and Colombia (Khazaei et al., 2018). Mélodie was bred at INRA (Institut National de la Recherche Agronomique), France and has high efficient use of water (Khan et al., 2010) and relatively high productivity (Khazaei et al., 2014). Inbred lines were selected from each parent and designated with the /2 suffix. Khan et al. (2007) reported that Mélodie/2 and ILB 938/2 had significantly higher WUE (3.2 g.L^{-1}) than Aurora (2.8 g.L^{-1}) under non-stress conditions.

The three crosses Aurora/2 \times Mélodie/2, ILB 938/2 \times Aurora/2, and Mélodie/2 \times ILB 938/2 were prepared by manual pollinations in the insect-proof glasshouse of the Department of Agricultural Sciences, the University of Helsinki, Finland. The standard sets of quantitative standard generations were prepared (P_1 , P_2 , F_1 , F_2 , B_1 , and B_2) for determination of modes of gene action for photosynthesis related traits. P_1 and P_2 refers to parental lines, F_1 single crosses, F_2 single crosses selfed, and B_1 and B_2 backcrosses with P_1 and P_2 , respectively.

The sample sizes (*i.e.* number of plants analysed) in each cross were: 20 plants each for P_1 and P_2 , 20 plants for F_1 , 100 plants for F_2 , and 20 plants each for B_1 and B_2 . Pots were randomized within each cross. Each pot contained one plant.

2.2 Growing conditions

Plants were grown in 2 L plastic pots in a climate-controlled glasshouse of the Department of Agricultural Sciences, University of Helsinki. The potting medium comprised 2 parts peat

(White 420 W, Kekkilä Oy, Vantaa, Finland) and 1 part sand (0.5–1.2 mm, Saint-Gobain Weber Oy Ab, Helsinki, Finland) (v:v). Seeds were inoculated with *Rhizobium leguminosarum* biovar. *viciae* (Elomestari Oy, Tornio, Finland). The photoperiod was adjusted to 14 h light and 10 h dark and photosynthetic photon flux density (PPFD) was $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the canopy level from artificial lighting. The temperature was set to 21°C day/15°C night and the relative humidity was maintained at 60%. Soil moisture level was maintained at field capacity with automatic irrigation during the experiments.

2.3 Measurements

Nine weeks after sowing, the following morpho-physiological measurements were taken on the youngest fully expanded leaves. At this time, most accessions had either open flowers or flower buds, so they were committed to flowering.

Chlorophyll content was measured using a SPAD (Soil Plant Analysis Development) 502 Plus Chlorophyll Meter (Konica Minolta Sensing, Inc., Japan). Measurements were recorded between 7:00 and 9:00 a.m. to minimize the potential effects of light intensity on chloroplast movement.

Leaf area was measured using a LI-3050C (LI-COR, Inc., Lincoln, NE, USA). Photosynthetic rate (A_{net}) and stomatal conductance (g_s) both were measured using a LI-6400 portable photosynthesis system (LI-COR, Inc.) equipped with a 2×3 cm leaf chamber with a LED light source (6400-02B, 90% red and 10% blue). Photosynthesis photon flux density (PPFD) was $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. A CO₂-injecting cartridge was attached to the system to control reference CO₂ concentration at $400 \mu\text{mol mol}^{-1}$. The flow rate was $400 \mu\text{mol s}^{-1}$. Measurements were done between 9:00 and 11:30 a.m. Gas exchange traits for each cross along with its quantitative standard generations was measured on a separate day.

Maximum quantum efficiency of photosystem II (PSII), F_v/F_m , and electron transfer rate (ETR) of PSII were measured using a PAM 2500 Portable Chlorophyll Fluorescence (Heinz Walz GmbH, Germany) (*see Schreiber et al., 2011*). ETR was given by the expression:

$$ETR = \Phi_{PSII} \times PPFD \times 0.5 \times 0.84.$$

Canopy temperature was measured using a FLUKE® 574 infrared thermometer gun (IRT, FLUKE, Everett, WA, USA). The instructions of using the IRT were followed to avoid false and non-repeatable results (Blum, 2011).

Biomass (above and below ground) was measured after harvesting 13-week-old plants and drying the samples for 48 h in a 60°C oven.

2.4. Genetic analysis

To test the accuracy of additive-dominance model, the scaling test was performed with the following equations: $A = 2 \overline{B_1} - \overline{P_1} - \overline{F_1}$, $B = 2 \overline{B_2} - \overline{P_1} - \overline{F_1}$, $C = 4 \overline{F_2} - 2 \overline{F_1} - \overline{P_1}$

– $\overline{P_2}$. Deviation from zero indicated that simple additive-dominance is inadequate and the presence of epistasis. The prospective variance and standard error (SE) were calculated for A, B and C scales. The t-test was performed by dividing calculated variance to the SE for each scale.

The means and variances were partitioned according to the weighted least-squares methods described in Mather & Jinks (1971) using their genetic coefficients (Table 1). Gamble (1962) notation was used in defining the parameters. Adjustment of the generation means to genetic models was tested using χ^2 tests. Coefficients for the genetic effects (Table 1) and the mean of six basic generations (P_1 , P_2 , F_1 , F_2 , B_1 and B_2) were sequentially adjusted to the model via weighted least squares and the best fit was determined with the simplest model that was found to be non-significant in the χ^2 test. The significance of the genetic parameters was tested with t-tests (Mather & Jinks, 1982; Kearsley & Pooni, 1996). The models were: model only with mean, an additive-dominance model (mean, additive and dominance), and additive-dominance models with epistasis (additive \times additive, additive \times dominance and dominance \times dominance). The estimated genetic parameters were: m = mid-parent value, a = additive gene effect, d = dominance gene effect, aa = additive \times additive epistatic effect, ad = additive \times dominance epistatic effect, and dd = dominance \times dominance epistatic effect. Genetic analysis was computed using MS Excel, 2017. Principal component analysis (PCA) was employed to illustrate relationships between photosynthesis-related measurements and quantitative standard generations using the R statistical package (R Development Core Team, 2016).

3 Results

The means and standard errors of the six standard generations on photosynthesis-related traits for three crosses are shown in Table 2. ILB 938/2 had smaller leaves, lower SPAD (yellow leaves), warmer canopy, lower photosynthetic rate, lower stomatal conductance, lower F_v/F_m , lower ETR and consequently lower biomass production than the other two parental lines. Aurora/2 had larger leaves and higher stomatal conductance and ETR than Mélodie/2. Heterosis (over best plant) for biomass was 31.0%, 7.7% and 2.2% for crosses Aurora/2 \times Mélodie/2, ILB 938/2 \times Aurora/2, and Mélodie/2 \times ILB 938/2, respectively.

PCA results revealed that the mean value of B_1 was close to P_1 and B_2 was close to P_2 , and F_1 was close to F_2 in all crosses (Figure 1 A, B, and C). In cross Aurora/2 \times Mélodie/2, principal component 1 (PC1) explained nearly 50% of the total trait variation, and the second principal component (PC2) explained 33% of the variation. PC1 separated only P_2 and B_2 from other generations. In both cross 2 (ILB 938/2 \times Aurora/2) and cross 3 (Mélodie/2 \times ILB 938/2), PC1 explained over 80% of the total variation, separating parental lines along with backcrosses from F_1 and F_2 generations. The PC2 accounted for 11% and 13% of the variation in cross 2 and cross 3, respectively. In all crosses, the F_1 generation had above-average biomass expression. SPAD and F_v/F_m were closely associated.

The scaling test results (Table 3) suggest that the simple additive-dominance model did not fit for most of measurements except for stomatal conductance and F_v/F_m in all three crosses. Additionally, scales A and B revealed a fit for canopy temperature in all crosses and for photosynthetic rate in cross Aurora/2 × Mélodie/2. Rejection of the additive-dominance model may indicate that epistasis and/or linked gene effects contributed to genetics of those traits.

In the cross Aurora/2 × Mélodie/2, both additive and dominance gene effects were only significant for leaf area (Table 4). SPAD was the only trait without a significant fit. The additive estimates were positive and significant for leaf area and stomatal conductance (P_1 had greater values than P_2) and negative and significant for SPAD and F_v/F_m (P_1 had smaller values than P_2). The dominance gene effect was important for leaf area and biomass and it was greater than estimates of additive gene effect particularly several magnitudes for biomass. The dominance estimate sign (+ or -) indicates the relation of the F_1 hybrid value to the mid-parent value and shows the contribution of parental lines to the dominance effects. For example, dominance gene effects for leaf area and biomass were contributed by the genes from Aurora/2 (parent with higher value). In the first cross, the additive × dominance interaction effect was significant for the canopy temperature and F_v/F_m .

In the cross ILB 938/2 × Aurora/2 the additive estimates were negative for all studied traits except canopy temperature, because P_1 (ILB 938/2) had smaller values than P_2 (Aurora/2) for those traits (Table 4). Additive gene effects were significant in all cases, whereas the dominance gene effect was important for leaf area, SPAD, ETR and biomass. For leaf area, SPAD, stomatal conductance, ETR and biomass the dominance gene effects in cross ILB 938/2 × Aurora/2 were contributed by the genes from Aurora/2 (parent with higher value). The additive × additive and additive × dominance interaction effects were significant for the leaf area and stomatal conductance, and SPAD, respectively in the second cross. Additionally, the dominance × dominance interaction effect was significant for the photosynthetic rate.

For the third cross, Mélodie/2 × ILB 938/2, additive and dominance gene effects were important for leaf area, canopy temperature, photosynthetic rate, stomatal conductance, F_v/F_m and biomass (Table 4). Additive gene effects alone were important for SPAD and F_v/F_m . The additive estimates were positive for all studied traits except canopy temperature, indicating that P_1 (Mélodie/2) had greater values than P_2 (ILB 938/2) for those traits in this cross. The positive sign for the dominance estimates of leaf area, SPAD, photosynthetic rate and biomass showed that dominance gene effects in this cross were contributed by the genes from Mélodie/2. The additive × additive and dominance × dominance interaction effects were significant for the ETR and stomatal conductance, respectively.

4 Discussion

Understanding the genetic basis of photosynthesis-related traits contributes to enhanced seed yield of faba bean. We created three crosses using three parental lines with distinct morpho-physiological characteristics related to photosynthesis. The dominance effects were generally

greater than additive effects, indicating that major genes are likely to affect the studied traits across the three crosses. Although the study identified the important gene effects controlling photosynthesis-related traits, it should be noted that these traits are highly influenced by environmental conditions (high G × E interaction), time of day, stage of plant development, and most importantly, water status in faba bean (Khan et al., 2007; Khazaei et al., 2013, 2014; Alghamdi et al., 2015) as in other legumes (Leport et al., 1998) and other crops (Tardieu, 2013).

Non-significant estimates of the additive and dominance effects in the cross Aurora/2 × Mélodie/2 for photosynthetic rate, canopy temperature and ETR could be explained by the lack of variability among generations for these characteristics, particularly between the parental lines. Both Aurora/2 and Mélodie/2 originate in Europe and were shown to have high genetic similarity by SNP (single nucleotide polymorphism) markers (Webb et al., 2016). The PCA results also showed that the parental lines were not separated by any photosynthesis-related measurements (Figure 1A). This research was carried out under non-stress conditions, so genotypes could show maximal performance. However, under water stress conditions, Aurora/2 and Mélodie/2 showed opposite responses, one being very sensitive and the other one tolerant (Khan et al., 2007). In the second and third crosses which included ILB 938/2 as one of the parental lines, a wider variability among generations were observed.

In bi-parental crosses, the less divergent the parental lines, the more likely the possibility of detecting important additive gene effects in the inheritance of quantitative traits. By increasing the diversity between parental lines, dominance and epistatic effects may play significant roles in the inheritance of quantitative traits (Halward & Wynne, 1991). In cross Aurora/2 × Mélodie/2 with little genetic diversity, there was a preponderance of additive genes for SPAD, stomatal conductance and F_v/F_m . In crosses ILB 938/2 × Aurora/2 and Mélodie/2 × ILB 938/2, for which the parental lines were more diverse (both genetically and morphologically), dominance effects played a significant role in the inheritance of several traits. Traits that are controlled mainly by additive gene action may be considered for selection at early generations, suggesting that more distantly related crosses (preferably between an exotic and a local germplasm) increase the speed and efficiency of breeding by early selection. These kinds of traits may be improved by direct selection after hybridization and by improvement using synthetic varieties. A similar strategy is in use in the faba bean and lentil breeding programs at the University of Saskatchewan with F_2 -derived breeding. In broader crosses, however, later generation selection may be rewarded by the appearance of novel gene combinations (Isleib & Wynne, 1983; Yang, 2009; van Ginkel & Ortiz, 2018).

Data presented here indicate that gene effects controlling most of studied traits varied in different crosses. Thus, a specific breeding strategy needs to be adapted for each cross. For example, for photosynthetic rate, no significant gene action effects were found in the first cross. However, the second and third cross indicated only additive and additive-dominance gene effects, respectively. It should be also noticed that faba bean is a partially allogamous crop species and breeding systems range between self- or cross-pollinating. All breeding methods may be used in the genetic improvement in this species. The three main breeding

strategies that have been largely employed are population development with recurrent selection, line breeding with management of the degree of cross-pollination by isolation strategies, and development of synthetic varieties (Gnanasambandam et al., 2012; Maalouf et al., 2018).

The large estimates of dominance effects for biomass, particularly in crosses Aurora/2 × Mélodie/2 and Mélodie/2 × ILB 938/2, indicate the importance of dominance gene effects in the inheritance of productivity in faba bean. This finding is in agreement with earlier reports (Hobbs & Burnett, 1982; Farag & Afiah, 2012; Abd El-Zaher, 2016) in this species. Selection may be delayed to later generations, which may provide an opportunity to further improve yield by *e.g.*, pedigree-based breeding system in segregating populations.

An F₅ recombinant inbred line (RIL) population of the third cross in this study (Mélodie/2 × ILB 938/2) has been genetically mapped for gas exchange traits and canopy temperature (Khazaei et al., 2014). A single QTL on chromosome 2 was identified for canopy temperature in well watered conditions. The results of the current study confirmed that this trait is controlled by few genes. QTL mapping results indicated that the alleles conferring warmer leaves under non-stress conditions were likely derived from Mélodie/2 and those conferring cooler leaves under water stress conditions were from ILB 938/2. Additionally, in the current study using generation mean analysis, the negative sign of dominance effect in the same cross, indicated that dominance effect was likely contributed by the parent having alleles responsible for cooler leaves (Mélodie/2) and were dominant over those of ILB 938/2. These findings reinforce the value of combining data on the molecular and conventional level to understand gene action.

In conclusion, this study indicates that selection for canopy temperature, SPAD and F_v/F_m could be done in the early generations, while selection efforts for productivity may be delayed to later generations. The results suggest that faba bean improvement programs may concentrate on selecting for canopy temperature and SPAD as non-destructive high-throughput and low-cost phenotyping tools for accelerated breeding for high photosynthetic efficiency in programs aiming to increase yield through physiological approaches. Increasing crop productivity through improved photosynthesis will require to be matched with increased carbon allocation to economic yield as well as maintenance of improved agronomy.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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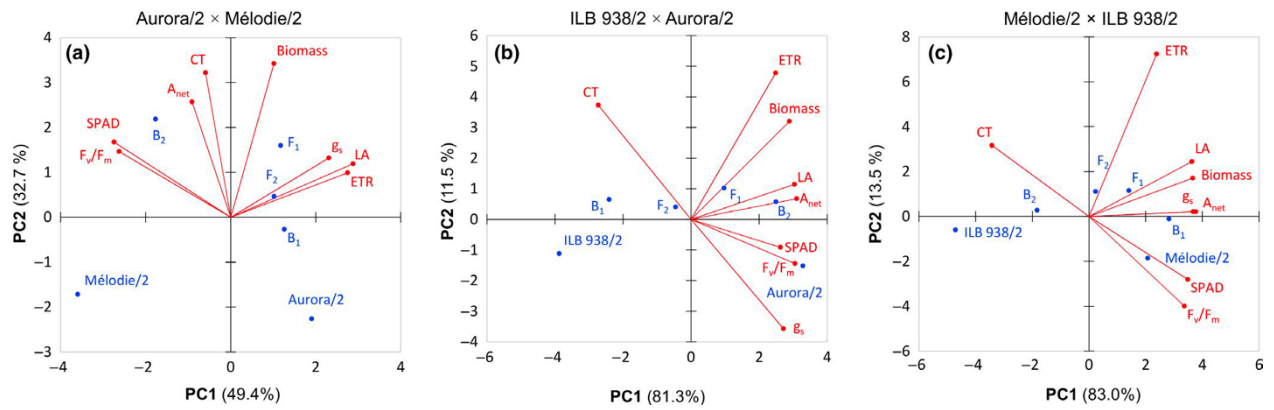


Figure 1. Principal component analysis bi-plots representing six standard sets of quantitative standard generations (P_1 , P_2 , F_1 , F_2 , B_1 , and B_2) and their relation to the photosynthesis-related measurements in faba bean three crosses. **A**, cross Aurora/2 \times Mélodie/2. **B**, cross ILB 938/2 \times Aurora/2. **C**, cross Mélodie/2 \times ILB 938/2.

Table 1. Genetic coefficients for the weighted least-square analysis of generation means

Generation	coefficients					
	<i>m</i>	<i>a</i>	<i>d</i>	<i>aa</i>	<i>ad</i>	<i>dd</i>
P_1	1	1	0	1	0	0
P_2	1	-1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	0.5	0	0	0.25
B_1	1	0	0.5	0.25	0.25	0.25
B_2	1	-0.5	0.5	0.25	-0.25	0.25

m, mean; *a*, additive; *d*, dominance; *aa*, additive \times additive; *ad*, additive \times dominance; *dd*, dominance \times dominance.

Table 2. Mean \pm standard error of six generations on different photosynthesis-related traits for three faba bean crosses

Cross and generation	LA (cm)	SPAD	CT (°C)	A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	ETR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	F_v/F_m	Biomass (g.plant ⁻¹)
<i>Aurora/2</i> \times <i>Mélodie/2</i>								
P ₁	70.05 \pm 1.30	33.38 \pm 0.42	17.94 \pm 0.11	10.62 \pm 0.15	0.532 \pm 0.018	19.18 \pm 1.45	0.689 \pm 0.002	8.53 \pm 0.25
P ₂	55.37 \pm 0.92	42.39 \pm 0.48	17.97 \pm 0.12	10.84 \pm 0.12	0.336 \pm 0.015	16.64 \pm 1.36	0.711 \pm 0.002	8.50 \pm 0.24
F ₁	76.04 \pm 1.32	37.98 \pm 0.53	18.24 \pm 0.14	10.92 \pm 0.12	0.491 \pm 0.015	20.00 \pm 1.63	0.702 \pm 0.003	11.17 \pm 0.29
F ₂	72.48 \pm 1.62	38.41 \pm 0.35	18.22 \pm 0.06	10.76 \pm 0.07	0.470 \pm 0.012	19.84 \pm 0.70	0.696 \pm 0.005	10.55 \pm 0.43
B ₁	73.36 \pm 1.77	36.57 \pm 0.35	17.86 \pm 0.09	11.03 \pm 0.10	0.490 \pm 0.015	18.57 \pm 1.58	0.686 \pm 0.003	10.35 \pm 0.41
B ₂	63.77 \pm 1.27	42.78 \pm 0.48	18.45 \pm 0.10	11.04 \pm 0.12	0.515 \pm 0.018	17.74 \pm 1.76	0.712 \pm 0.003	10.51 \pm 0.41
<i>ILB 938/2</i> \times <i>Aurora/2</i>								
P ₁	25.90 \pm 1.34	25.81 \pm 0.34	20.11 \pm 0.07	5.96 \pm 0.42	0.179 \pm 0.014	14.39 \pm 1.04	0.628 \pm 0.008	2.26 \pm 0.15
P ₂	70.05 \pm 1.30	33.38 \pm 0.42	18.19 \pm 0.04	11.52 \pm 0.15	0.434 \pm 0.014	21.42 \pm 1.74	0.670 \pm 0.002	8.53 \pm 0.25
F ₁	67.69 \pm 1.94	27.49 \pm 0.59	19.68 \pm 0.14	10.64 \pm 0.28	0.266 \pm 0.014	23.58 \pm 2.30	0.651 \pm 0.006	9.19 \pm 0.27
F ₂	48.13 \pm 1.48	31.16 \pm 0.36	20.18 \pm 0.06	8.97 \pm 0.21	0.206 \pm 0.007	20.37 \pm 0.90	0.648 \pm 0.004	7.08 \pm 0.24
B ₁	36.25 \pm 2.34	28.08 \pm 0.63	20.68 \pm 0.09	6.79 \pm 0.48	0.203 \pm 0.019	20.66 \pm 1.45	0.627 \pm 0.007	5.40 \pm 0.36
B ₂	64.48 \pm 1.90	33.42 \pm 0.51	18.86 \pm 0.11	11.09 \pm 0.16	0.290 \pm 0.014	26.32 \pm 1.62	0.659 \pm 0.005	9.23 \pm 0.32
<i>Mélodie/2</i> \times <i>ILB 938/2</i>								
P ₁	55.37 \pm 0.92	42.39 \pm 0.48	17.97 \pm 0.12	11.91 \pm 0.08	0.245 \pm 0.011	15.00 \pm 1.51	0.708 \pm 0.004	8.50 \pm 0.24
P ₂	25.90 \pm 1.34	25.81 \pm 0.34	19.42 \pm 0.14	6.44 \pm 0.28	0.130 \pm 0.009	12.63 \pm 1.09	0.636 \pm 0.010	2.26 \pm 0.15
F ₁	65.09 \pm 1.72	34.19 \pm 0.40	18.44 \pm 0.25	11.55 \pm 0.14	0.257 \pm 0.011	20.96 \pm 1.63	0.665 \pm 0.011	8.69 \pm 0.64
F ₂	55.56 \pm 1.34	35.20 \pm 0.44	18.98 \pm 0.08	10.52 \pm 0.18	0.202 \pm 0.007	20.97 \pm 0.66	0.668 \pm 0.004	8.01 \pm 0.24
B ₁	69.91 \pm 2.49	41.02 \pm 0.57	18.18 \pm 0.16	12.08 \pm 0.14	0.271 \pm 0.014	19.64 \pm 1.51	0.700 \pm 0.003	9.28 \pm 0.28
B ₂	42.80 \pm 2.84	33.29 \pm 1.22	19.38 \pm 0.16	8.54 \pm 0.51	0.162 \pm 0.017	16.95 \pm 1.76	0.658 \pm 0.012	6.44 \pm 0.42

LA, leaf area; *CT*, canopy temperature; *A_{net}*, photosynthetic rate; *g_s*, stomatal conductance; *ETR*, electron transfer rate of PSII; *F_v/F_m*, maximum quantum efficiency of photosystem II (PSII).

Table 3. Estimates of scaling tests (along with t-value) on different photosynthesis-related traits for three faba bean crosses

Cross and scale	LA	SPAD	CT	A _{net}	g _s	ETR	F _v /F _m	Biomass
<i>Aurora/2 × Mélodie/2</i>								
A	0.63 (17.34)***	1.78 (4.16)***	-0.46 (1.09)	0.53 (1.22)	-0.04 (0.17)	-2.05 (16.51)***	-0.02 (0.03)	0.99 (3.92)***
B	-3.88 (13.37)***	5.20 (5.10)***	0.69 (1.11)	0.32 (1.33)	0.20 (0.19)	-0.98 (17.89)***	0.01 (0.03)	1.34 (3.85)**
C	12.42 (64.43)***	1.90 (14.83)***	0.50 (2.88)**	-0.25 (3.17)***	0.03 (0.50)	3.72 (32.22)***	-0.02 (0.21)	2.84 (16.97)***
<i>ILB 938/2 × Aurora/2</i>								
A	-21.10 (22.50)***	2.86 (6.02)***	1.58 (0.86)	-3.01 (4.67)***	-0.04 (0.19)	3.34 (15.25)***	-0.02 (0.07)	-0.64 (3.42)***
B	-8.77 (18.90)***	5.96 (5.06)***	-0.14 (1.08)	0.02 (2.37)**	-0.12 (0.15)	7.63 (16.47)***	0.00 (0.06)	0.74 (3.16)**
C	-38.82 (60.09)***	10.47 (15.40)***	3.06 (2.70)**	-2.87 (9.05)***	-0.32 (0.33)	-1.49 (41.64)***	-0.01 (0.18)	-0.84 (9.98)***
<i>Mélodie/2 × ILB 938/2</i>								
A	19.35 (18.65)***	5.46 (4.45)***	-0.05 (1.44)	0.71 (1.65)	0.04 (0.12)	3.31 (12.84)***	0.02 (0.05)	1.37 (3.70)***
B	-5.39 (20.96)***	6.59 (8.48)***	0.89 (1.39)	-0.92 (3.78)***	-0.06 (0.13)	0.31 (15.03)***	0.01 (0.10)	1.93 (3.13)***
C	10.78 (55.19)***	4.23 (17.05)***	1.66 (3.88)***	0.65 (7.45)***	-0.08 (0.31)	14.33 (30.09)***	-0.00 (0.18)	3.88 (10.48)***

LA, leaf area; CT, canopy temperature; A_{net}, photosynthetic rate; g_s, stomatal conductance; ETR, electron transfer rate of PSII; F_v/F_m, maximum quantum efficiency of photosystem II (PSII).

** and ***, P < 0.01 and 0.001, respectively.

Table 4. Estimates of mean (m), additive (a), dominance (d) and epistatic (aa, ad and dd) genetic effects \pm standard error on different photosynthesis-related traits for three faba bean crosses
LA, leaf area; *CT*, canopy temperature; *A_{net}*, photosynthetic rate; *g_s*, stomatal conductance; *ETR*, electron transfer rate of PSII; *F_v/F_m*, maximum quantum efficiency of photosystem II (PSII).

*, ** and ***, $P < 0.05$, 0.01 and 0.001 , respectively. *ns*; non-significant.

Cross and gene effect	LA	SPAD	CT	A _{net}	g _s	ETR	F _v /F _m	Biomass
<i>Aurora/2</i> × <i>Mélo die/2</i>								
m	62.84±0.75***	33.65±1.83*	18.14±0.04***	10.86±0.04***	0.434±0.012***	19.06±0.49***	0.700±0.001***	8.63±0.17***
a	7.71±0.75**	-4.90±0.28*			0.098±0.012**		-0.011±0.002**	0.01±0.17
d	13.23±1.46**	14.70±4.53			0.152±0.051			2.84±0.32**
aa		4.29±1.80						
ad			-1.17±0.27		-0.236±0.053		-0.031±0.010*	
dd		-10.37±2.93			-0.095±0.050			
χ ² (df)	5.80 ns (3)	6.49** (1)	7.52 ns (4)	10.40 ns (5)	3.66 ns (1)	5.90 ns (5)	1.70 ns (3)	4.69 ns (4)
<i>ILB 938/2</i> × <i>Aurora/2</i>								
m	27.72±3.46*	29.62±0.27***	20.69±0.18**	8.58±0.16***	0.146±0.020*	18.24±0.93***	0.647±0.002***	5.40±0.14***
a	-22.56±0.89***	-3.93±0.26***	0.96±0.04*	-2.90±0.19**	-0.121±0.009*	-3.90±0.91*	-0.023±0.003**	-3.19±0.14***
d	39.33±4.87**	7.81±1.47*	-1.02±0.30		0.119±0.031	6.36±2.00*		3.72±0.28***
aa	20.09±3.60*		-1.54±0.19		0.160±0.022*			
ad		-9.94±1.69*	1.71±0.31	-2.37±0.71				
dd				1.98±0.35*				
χ ² (df)	5.20 ns (2)	3.52 ns (2)	0.08 ns (1)	1.09 ns (2)	2.56 ns (2)	5.72 ns (3)	2.19 ns (4)	2.63 ns (3)
<i>Mélo die/2</i> × <i>ILB 938/2</i>								
m	41.10±0.78***	26.95±2.77	19.52±0.29***	9.19±0.13***	0.190±0.006***	20.87±0.57***	0.673±0.003***	5.37±0.14***
a	14.57±0.81***	8.26±0.29*	-0.79±0.09**	2.77±0.13***	0.063±0.007**		0.039±0.004***	3.11±0.14***
d	26.07±1.73***	25.75±7.32	-1.08±0.50	2.50±0.19***				2.03±1.08**
aa		7.14±2.74	-0.82±0.31			-7.50±1.07**		
ad	26.25 ±7.66							
dd		-18.52±4.68			0.068±0.013*			-3.71±1.51
χ ² (df)	4.30 ns (2)	0.17 ns (1)	3.63 ns (2)	6.07 ns (3)	6.93 ns (3)	3.20 ns (4)	7.24 ns (4)	0.28 ns (2)