

Background

Insufficiency of pollination can be a major constraint to the potential yield in faba bean, due to lack of autofertility or lack of pollinator activity. Faba bean is a partially allogamous plant. Both self and cross fertilization happen. Cross fertilization fully depends on pollinator activity while self-fertilization can occur by pollinators or by spontaneous selfing. Honey bees (*Apis mellifera*), bumble bees (*Bombus* sp.) and solitary bees visit the flower and have a role as pollinator.

The ability of a plant to self-fertilize without pollinators and without external mechanical stimulus in faba bean is named autofertility. The flower of faba bean has complete reproduction organs but in most genotypes spontaneous self-fertilization is incomplete. Some reports show that each of stigma, style and pollen have an important impact on autofertility.

The purpose of the current experiments is to study the genetic variability of reproductive features in detail and to identify QTL responsible for variation of this trait.



Material and Methods

Plant materials

189 lines of winter faba bean population, developed in Göttingen since 1989 from 11 founder lines (inbred winter faba bean lines). The 11 founder lines were mixed to produce a recombining population. After nine generations, around 400 lines were obtained and named Göttingen winter bean population (GWBP). The 189 lines were randomly selected from GWBP and bred via single seed descent.



Fig.1 The experiments in bee isolation houses

Phenotypic data

The plants were grown in bee-isolation houses covering 24.5 m² area each. The trials were conducted in alpha-lattice design with two replications. Plants were standardized according to Table 1.

Table 1. The standardization scheme

Year	Tillers	Inflorences (infl.) per tiller	Flowers per infl.
2013	3 tillers	10 inflorences (infl.) per tiller	2 flowers per infl.
2014	2 tillers	8 infl. in the first tiller and 4 infl. in the second tiller	
2015	3 tillers	10 infl. per tiller	
	2 tillers	8 infl. in the first tiller and 4 infl. in the second tiller	
	2 tillers	Max infl. per tiller	

The experiments were conducted using two treatments, tripping and non-tripping. Tripping is the manual substitute for the pollinator-mediated, mechanical stimulus of fertilization.

Association Studies

Association analysis was conducted using TASSEL 3.0. A total of 2017 markers consisted of 1828 AFLP markers and 189 SNP markers were employed, which 1136 markers showed polymorphism with a minimum allele frequency of 10% in the faba bean lines. Mixed linear model (MLM) with kinship matrix were used. A false discovery rate (FDR) of 20% was used to test the statistical significance of markers-traits associations.

Results

Large genetic variation was uncovered within the faba bean genotypes in tripped and un-tripped treatment. Heritability for all of the traits after treatment in tripping and non-tripping was medium to high (data not shown).

The autofertility could be observed with rate of fertilization, which indicates the fertilization level of the genotype. Obviously, tripping increased the rate of fertilization. The rate of fertilization was quite similar in two seasons (Fig. 2) for both treatments showed that the treatments were reproducible. The autofertility variation clearly be seen in non-tripping treatment among the genotypes.

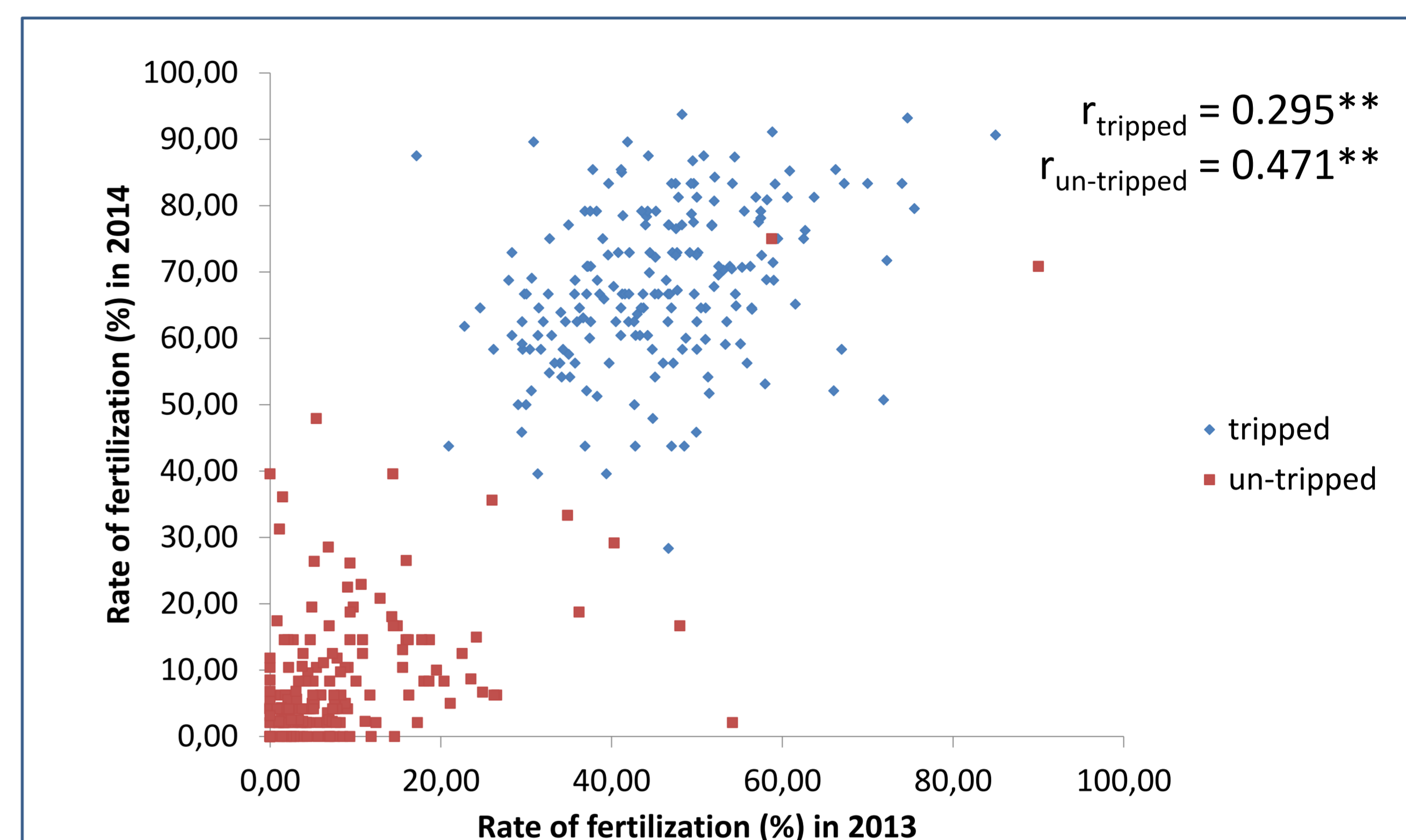


Fig.2 Rate of fertilization of faba bean genotypes in season 2013 and 2014 of tripping and non-tripping treatments

Several significant putative marker for QTL connected to several traits were identified in the preliminary association mapping (Tab. 2). Some markers have relation with more than one trait, like E36M56-229 and Vf_Mt4g068010_001. The association analysis is still ongoing for some traits that will provide more results to strengthen the current understanding of reproductive features in faba bean.

Table 2. Preliminary association mapping results of faba bean genotypes for reproductivity features

Trait	Marker	LG*	Polymorfism	Position (cM)*	marker p	marker R ²
Plant height	E36M56-229	4	0:1	168.6	0.0000	0.1202
First flower position	E36M56-229	4	0:1	168.6	0.0005	0.0752
	E40M55-299	6	1:0	47.1	0.0000	0.0991
Flowering time	Vf_Mt4g068010_001	7	G:C	104.0	0.0004	0.0854
	Vf_Mt4g068010_001	7	G:C	104.0	0.0001	0.1015
Rate of Fertilization	W3258243	-	1:0	-	0.0002	0.0772

* Based on Gregor Welna (2014)

Conclusion

High amount of genetic variation were found for the observed traits. Our study shows promising results for identifying putative markers which related to some reproductive features in faba bean. Furthermore the findings could give benefit to develop marker based assisted selection in faba bean.

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