Faba bean takes the spotlight

Current faba bean research at Göttingen, Germany

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Abstract: Faba bean breeding research at Göttingen has been held for over five decades. Current projects include studies on freezing tolerance, pollinators and reproductive behavior, seed-weight variance, as well as identifying highly inbred individuals existing a priori in faba bean populations. The focus of the projects is on plant breeding methodology, to improve future breeding schemes.

Key words: breeding methodology, inbred lines, QTL, synthetics, seed weight, winter hardiness

The grain legume faba bean is cultivated as both a spring- and autumn-sown crop in Europe. While in the Mediterranean Basin, faba beans are typically sown in autumn to exploit the rain-fed winter months, they are mainly cultivated as spring crop north of the Alps. The genuine winter faba bean is only cultivated in North-West (NW) Europe. Mild winter conditions favor large-scale winter faba bean production in the UK and France, where winter beans accounted for approximately 40% (75,000 ha) and 75% (51,308 ha) of the respective total faba bean cultivation area in 2022 [1]. Despite the yield advantage over the spring-type, the cultivation of winter faba bean remains limited in other countries of NW Europe due to the insufficient winter hardiness of current cultivars. Consequently, breeding for improved winter hardiness is of great importance to facilitate local plant protein production in NW European countries, like Germany. One substantial aspect of winter hardiness is tolerance against frost

in winter and against frost in early spring (late-frost), which occurs in early spring when plants have lost their cold acclimation, i.e., are dehardened.

In our ongoing project, we have adapted the sophisticated climate chamber frost stress protocol, initially developed in 2003 and subsequently refined [2,3,4], to included late-frost treatments on dehardened plants [5]. We tested a broad panel of inbred lines, most derived from the "Göttingen Winter Bean Population" plus further diverse types. The lines were exposed to frost in a climate chamber and phenotyped. Utilizing recent developments in faba bean genomics, such as the 60K Vfaba_v2 Axiom SNP array [6] and the V. faba reference genome, we identified via genome-wide associations studies (GWAS) multiple validated quantitative trait loci (QTL) associated with tolerance against frost and late-frost, e.g., for freezing damage (Figure 1) and survival after frost. Currently, we are developing genomic prediction models for winter-frost and late-frost tolerance as well as field-based traits such as yield.

Faba bean is a partially allogamous species. European faba bean cultivars are usually synthetic populations which utilize part of the het-

Figure 1. Differentiation for frost damage among four different winter faba bean inbred lines on juvenile potted plants after frost stress in a climate chamber experiment. Shown are two plants per inbred line and pot corner. Frost damage is visible as loss of leaf turgidity and loss of color in leaf and stem (blackening of tissue).

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erosis. As yield and yield stability are improved in heterozygous and heterogeneous crop stands, increasing the share of heterosis in synthetic cultivars is a promising breeding strategy. High rates of cross-fertilization increase the share of heterosis realized in synthetic cultivars. Hence, the degree of cross-fertilization is a crucial parameter in breeders' yield predictions and used to predict inbreeding and share of heterosis. Available yield predictions assume that all genotypes contribute equally to the cross-fertilized seeds. We found genotypes to significantly and markedly differ in success rates as pollen donors [7]. Yet, allowing such differences in paternal outcrossing success in a population increases its inbreeding, thus decreases yield. Hence, differences in paternal success should be avoided.

To circumvent expensive paternity assessments, reproductive traits that are easier to assess were estimated for the same sixteen genotypes. Pollen production and pollen viability were highly correlated with paternal success in inbred lines (Brünjes and Link, under preparation). Hence, these traits could be used as proxy for paternal outcrossing success when selecting inbred lines as components of a synthetic.

As higher degrees of cross-fertilization improve seed yield in the next generation, we studied the pollination efficiency of individual bee species to pollinate faba bean. The most efficient pollinator species that enhanced cross-fertilization and seed set was *Bombus hortorum*, a less frequent and more specialized pollinator species with long head and long tongue (Figure 2). It effectuated 45% cross-fertilization, while the other species including honeybee realized less than 20% cross-fertilization each [8]. Thus, synthetic populations should be propagated in environments with large and vital *B. hortorum* populations, to maximize the genotype-specific degree of cross-fertilization and realize higher heterozygosity in subsequent generations, to better manage and exploit heterosis in faba bean.

To compose a synthetic, inbred lines are used. To develop them is tedious, as no doubled haploid technique is available, and faba beans need manual 'tripping' and bee-proof cages for selfing. Yet due to its mixed mating, faba bean pop-

ulations a priori contain individuals with very different inbreeding status, including highly inbred individuals (expectedly > 5%). We use KASP SNP markers and the 60K Vfaba_v2 Axiom SNP array [6] to investigate and exploit this situation within the 'Göttingen Winter Bean Population'. Moreover, Genomic selection will be applied to 'chipped seed'; a non-destructive method to extract DNA from faba bean seed cotyledons. In the end, the use of inbred lines in both line and population breeding will be facilitated to optimize breeding schemes [9].

The huge differences in faba bean's seed size made us analyse this domestication trait. First, from a cross of two lines differing strongly in seed weight, 'HediLin' (Thousand seed weight (TSW) 350 g) × 'PietraLin' (TSW 3000 g), we developed two near isogenic lines (NILs), one small-seeded and one large-seeded. These NILs are much more similar in growth and development than the initial parents, but still show an about two-fold difference in seed weight. Crossing these two NILs, we obtained N=180 recombinant inbred lines that were used to map

Table 1. QTL for seed weight in faba bean mapped in our RIL population.

	Mapped QTL				Effects		Confidence intervals		
No.	QTL	LG	Position [cM]	LOD	Add. [mg]	Variance explained [%]	[b]Low [cM]	[b]Up [cM]	[b]Size [cM]
1	sw1c.1AB	c.1AB	1.40	16.2	-36.2	6.6	0.56	2.24	1.68
2	sw1c.1CDsgl	c.1CDsgl	38.93	36.8	-72.6	20.3	38.08	39.49	1.41
3	sw2c.1CDsgl	c.1CDsgl	57.49	13.9	-37.2	5.5	56.64	63.00	6.36
4	sw1c.1EF	c.1EF	34.33	8.5	-25.2	3.1	28.06	38.82	10.75
5	sw1c.1GHI	c.1GHI	5.91	12.2	-30.8	4.7	4.00	7.32	3.32
6	sw2c.1GHI	c.1GHI	40.25	7.6	+14.0[a]	2.8	36.00	44.18	8.18
7	sw1c.2J	c.2J	1.00	12.0	-30.6	4.6	0.00	3.40	3.40
8	sw1c.4N	c.4N	13.52	19.7	-40.3	8.5	12.39	14.91	2.52
9	sw2c.5RSTU	c.5RSTU	4.00	6.6	-25.9	2.4	1.40	6.73	5.33
10	sw1c.5RSTU	c.5RSTU	17.98	12.2	-32.9	4.7	15.18	18.83	3.64
	Full model	-	-	79.5	-	87.4	-	-	-

LG: linkage group, c.1 to c.5 indicate the chromosomes to which the linkage groups belong, A to U refer to the segments polymorphic between the NIL parents of the mapping population that make up the linkage groups.
LOD: logarithm of the odds showing the statistical probability (10^{LOD}) of the QTL being real.

[a] the positive sign indicates that the allele of the small-seed parent increases seed size; [b] Low = Lower confidence interval, Up = Upper confidence interval, and Size of LOD 2.0 support intervals for the QTL positions

Figure 2. Long-tongued *Bombus hortorum*, the most efficient pollinator among five local bee species at Göttingen.

ten QTLs for seed weight. For nine of them, the allele of the large-seeded parent 'PietraLin' increased seed weight, yet, for one QTL (Table 1), the 'increase' allele came from small-seeded 'HediLin'. With a model including all 10 QTL, we could explain 87.4% of the phenotypic variance for seed weight in this cross. Hence, our QTL mapping captured the majority of the seed-weight loci.

In the past decades, only the Georg-August University Göttingen (www.uni-goettingen.de/de/48273.html) was home to a German academic group working on faba bean breeding. Recently, new groups in Germany joined faba bean research (University Giessen, JKI Quedlinburg, IPK Gatersleben) meaning good prospects for progress in research and in farmers' fields.

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