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Genetic analysis of winter hardiness and effect of sowing date on yield traits in winter faba bean

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ABSTRACT

Faba bean is an important grain legume crop that provides nitrogen input into temperate agriculture systems. Breeding for improving winter hardiness and frost tolerance of winter faba bean is currently needed. The main objectives of this study were to examine the winter hardiness and effect of sowing dates (20th October and 20th November of 2012) in a set of 20 genotypes (10 tolerant and 10 susceptible), selected from artificial frost stress experiments, under field conditions and to test the genetic diversity and validate some SNP associated with frost tolerance in the elite genotypes. The genotypes were selected, based on their regrowth after frost (REG, frost tolerance), from 189 winter faba bean genotypes evaluated in Frost Growth Chamber (FGCh). Two winter hardiness traits (winter survival rate and leaf frost susceptibility) and four yield traits (plant height, the number of pods, 100-seed weight, and days to maturity) were scored in this study. All frost-tolerant genotypes showed higher winter survival rate (85%) than the frost-susceptible group (67%) in the first sowing date, while, all genotypes survived in the second sowing date.. The effect of sowing date was not significant for only 100-seed weight. The genetic distance (GD) between genotypes was calculated using 189 single nucleotide polymorphism (SNPs). The GD ranged from 0.59 to 0.89, indicating that a high genetic diversity resided among genotypes. The results suggest that the first sowing date is better for high agronomic features, and selection should be performed to increase winter hardiness in winter faba bean genotypes. Among all SNPs tested in this study, one SNP was validated and found to be associated with increased winter survival rate and decreased leaf frost susceptibility.

1. Introduction

Fabe bean (*Vicia Fabe*. L) is a major grain legume crop and ranked fourth cool-season legume crop after peas, chickpeas and lentils. Due to its high protein content, faba bean is used for feeding animals in Europe. Winter faba bean has several advantages over the spring type including higher yielding and better use of soil moisture (Link et al., 2010). Various environmental factors affect winter faba bean growth in cool temperate climate (Arbaoui and Link, 2007). Frost stress is among these factors that cause a significant reduction in faba bean yield (Flores et al., 2012). The winter-hardy genotype depends on its tolerance to frost and other adverse abiotic and biotic conditions (Stoddard et al., 2006). The insufficient winter hardiness of the current faba bean germplasm limits its exploitation in food and agriculture. Therefore, breeding for improving winter hardiness and frost tolerance of winter faba bean is currently required.

Faba bean can tolerate cold temperature at a range extending from −6 °C for spring types to −12 °C for Côte d'Or (French winter cultivar) (Link et al., 2010). Overwintering, winter survival rate, leaf frost susceptibility, and field survival index were previously used to evaluate winter hardiness in grain legumes (Fowler and Gusta 1979; Arbaoui et al., 2008; Sallam et al., 2016a,b). Frost tolerance in winter faba bean can be assessed under freezing-controlled conditions which are simple, rapid, reliable, and allow replication of experiments over time (Arbaoui and Link 2007; Maqbool et al., 2009; Sallam et al., 2015). However, it is recommended to evaluate winter faba bean under both controlled and field conditions. Ordás López et al. (2006) suggested to select the best genotypes for cold tolerance under controlled conditions (frost growth chambers) and then select the best frost tolerant genotypes in the field.

Sowing date affects winter hardiness and yield attributes. Early sowing of winter faba bean may result in a vigorous winter growth. However, it may cause frost damage and leaf diseases (Greenwood, 1955). Faba bean plants showed a high survival rate in late sowing season (Annicchiarico and Iannucci, 2007). Basically, the best sowing

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date for increasing winter hardiness relies on the conditions within the target region and plants should be strong and of a deep root system at the time of frost (Summerfield, 1988). Few studies have been conducted on the effect of sowing date on winter hardiness and yield attributes in winter faba bean. Therefore, the objectives of the current study were (1) to examine the performance of elite genotypes, which were selected from artificial frost test, under field conditions, (2) to study the genetic diversity among the elite genotypes and validate some QTL associated with frost tolerance in the current plant materials, and (3) to study the effect of sowing dates (optimum and late sowing dates) on yield attributes.

2. Material and methods

2.1. Plant material

This study included a set of 18 highly homozygous lines selected among 189 Gottingen winter faba bean population, which derived from natural crossing between 11 founder lines, (GWBP – Gasim et al., 2004) and two recombinant inbred lines (RILs) derived from a cross between two frost tolerant inbred lines (Côte d'Or/1 (French inbred line) × BeanPureLine/4628 (BPL 4628, Chinese inbred line) (Arbaoui, 2007). See Table 1.

2.2. Artificial frost test

Initially, 189 and two RILs genotypes were evaluated for frost tolerance in Frost Growth Chamber in 10 replications (season 2011/2012) according to the protocol published in Sallam et al. (2015). The most 10 frost tolerant and 10 frost susceptible genotypes were then selected based on their regrowth after frost (REG, Sallam et al., 2015). In summary, the seedlings of 189 GWBP population along with the two RILs were exposed to hardening phase on 5 °C for 10 days and then to frost stress at −16, −18, −20 °C for three nights. After frost test, all plants were cut off at the second internode in order to test their ability to regrow. Regrowth after frost was scored by weighing the leaves of the surviving genotypes after cutting off them again. The highest 10 genotypes as well as the lowest 10 genotypes for regrowth after frost were selected (Table 1).

Table 1
list of genotypes which were based on their frost tolerance (regrowth after frost, REG).

Genotype	Pedigree[a]	Regrowth after frost (g)	Characterization
S_145	GWBP	2.96	Tolerant
S_004	GWBP	2.69	Tolerant
S_081	GWBP	1.97	Tolerant
S_151	GWBP	1.82	Tolerant
S_299	GWBP	1.8	Tolerant
S_028	GWBP	1.79	Tolerant
CB-18	Côte d'Or 1 × BPL 4628	1.78	Tolerant
S_291	GWBP	1.72	Tolerant
CB-95	Côte d'Or 1 × BPL 4628	1.65	Tolerant
S_271	GWBP	1.65	Tolerant
S_165	GWBP	0.28	Susceptible
S_129	GWBP	0.26	Susceptible
S_013	GWBP	0.19	Susceptible
S_111	GWBP	0.14	Susceptible
S_034	GWBP	0.11	Susceptible
S_235	GWBP	0.10	Susceptible
S_232	GWBP	0.09	Susceptible
S_070	GWBP	0.08	Susceptible
S_242	GWBP	0.05	Susceptible
S_030	GWBP	0.04	Susceptible

[a] See Material and methods.

2.3. Experimental layout

All the 20 genotypes were sown at the Experimental Field Station at the University of Göttingen, Germany (51°29′51.68″ N, 9°55′47.76″ E) in season 2012–2013. The genotypes were sown in two sowing dates (optimum sowing date on October 21, 2012, and late sowing date on November 21, 2012). The experimental design was a randomized complete block design with four replications in each sowing date. The minimum daily temperatures at the experimental site from the first sowing date till the end of frost is presented in Fig. 1. Fifteen seeds/genotype were sown in rows with 35 cm distance apart and 15 cm between seeds.

All genotypes were only exposed to freezing temperature in the first sowing date, while, all genotypes did emerge after frost period in the second sowing date. In the first sowing date, two winter hardiness traits were scored: (1) winter survival rate (WSR%) was calculated by dividing the number of surviving plants after the frost period by the number of emerged plants after sowing, (2) leaf frost susceptibility (LFS) was scored as a combination of loss of leaf turgidity and color in a scale ranging from 1 (full turgor + green leaves) to 9 (no turgor + black leaves). This trait was scored three times during frost (see Fig. 1). Then, all scores were averaged to form one trait leaf frost susceptibility (LFS). In both sowing dates, four yield traits were scored on each genotype: (1) plant height (pH, cm), (2) 100-seed weight, (3) number of pods/plant (NPOD), and (4) days to maturity (DTM) was calculated from the date of sowing until the date of which 50% of plants/genotype lost their green color. Exceptionally, WSR and LFS were scored on genotypes sown in six replications, while, all yield traits were scored in four replications in the two sowing dates.

2.4. Statistical analysis

The winter survival rate and leaf frost susceptibility in the first sowing date were analyzed using analysis of variance (ANOVA) using the following model

$$Yij = \mu + gi + rj + grij$$

where Yij is an observation of genotype *i* in replication *j*, μ is the general mean; *gi*, *rj* are the main effects of genotypes and replications, respectively; *grij* is genotype × replication interaction of genotype *i* with replication *j*. The data on these two traits were recorded on genotypes sown in six replications.

All yield traits in both sowing dates were analyzed using the following model

$$Y_{ijk} = \mu + g_i + r_j + s_k + gr_{ij} + gs_{ik} + grs_{ijk}$$

where Y_{ijk} is an observation of genotype *i* in replication *j* in sowing date *k*, μ is the general mean; g_i, r_j, s_k are the main effects of genotypes, replications, and sowing dates respectively; gr_{ij} is genotype × replication interaction of genotype *i* with replication *j*. gs_{ik} is the genotype × sowing date interaction of genotype *i* with sowing date *k*. grs_{ijk} is genotype × replication × sowing interaction. The yield attributed were scored on all genotypes which were sown in four replications in each sowing date.

All analyses were performed using R software (2013) and BlAPSTAT (Utz, 1991). The graphical analyses were produced by R software and Microsoft Excel 2013. Reputability estimates of the genotypes for each trait were calculated as (h^2 = genotypic variance/phenotypic variance).

2.5. Genetic analysis and QTL validation for frost tolerance and winter hardiness

DNA was extracted from the 20 genotypes of winter faba bean using illustra Nucleon PhytoPure Genomic DNA Extraction kits (GE