

## AGRONOMIC PERFORMANCE AND THE EFFECT OF SELF-FERTILIZATION ON GERMAN WINTER FABA BEANS

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### ABSTRACT

Faba bean is mostly grown as spring crop in Central Europe; additionally it is grown as winter crop in few mild areas of UK and France. A genetically diverse winter bean population selected for high winter hardiness and very promising as winter crop in Germany was studied here. The population was assessed for its agronomic performance as well as for the effect of inbreeding depression due to selfing using an open-pollinated generation and two inbred generations developed from it. The assessment was carried out at four environments in two successive seasons (2001 and 2002) at Goettingen, Central Germany. The open-pollinated population showed better performance in overwintering ability than the UK checks but equivalent performance in yield and other agronomic traits. Winter damage, plant height and yield suffered from inbreeding depression proving heterosis in the open-pollinated generation. Overwintering ability and yield were shown to exhibit a mid parent heterosis of about 32% and of about 75%, respectively. Application of the results in breeding of synthetic cultivars is discussed.

**Key Words:** breeding, winter faba beans, winter hardiness, yield, inbreeding depression, heterosis, synthetic cultivars

## INTRODUCTION

Faba bean (*Vicia faba* L.) is a partially allogamous grain legume grown for its high protein content in the seed. The edible protein in the seed is used for human and animal consumption. The crop contributes to soil fertility through biological N-fixation. Since 2001, cropping of faba bean in Europe has been enforced due to organic farming demand, interest in poultry feed and as a replacement for pea in soils infected by *Aphanomyces* [4]. Today, cropping of grain legumes is increasingly encouraged because of recent ban on the use of meat and bone meal for animal feed [22].

In Central and North Europe, faba bean is grown as spring crop; additionally it is grown as winter crop in mild areas of UK and France. In the UK, winter beans have been grown at least since 1825 [1]. Today, cultivars like Punch, Target, Clipper, Striker, Bulldog, Silver and Wizard are well known in practical farming. Winter beans are sown, do flower and are harvested earlier (early August) than spring beans and as a result give the farmer more time after harvest to grow a subsequent soil-covering crop during the following winter, thereby at least reducing leaching of nitrogen to the ground water [7]. Moreover, winter beans partly escape dry periods in June and July hence suffer less from drought stress compared to spring beans [17], [1]. Based on such advantages, winter faba bean is considered as one of the best potential sources of vegetable protein for animal feed in Europe.

In Germany, due to hard winter conditions, winter bean is not yet introduced in practical farming (von Kittlitz 1974, unpublished). However, through intensive breeding, Littman [17] developed the German cultivars, Wibio, Webo and Hiverna. Webo was registered by the "Beschreibende Sortenliste" as "recommended for autumn sowing" in 1979 and Hiverna in 1986. Although these cultivars were believed to possess a winter hardiness equal to the winter form of other winter crops (e.g. winter oats, winter barley) and higher yield than spring beans [17], empirical data do only partly substantiate this; efforts to extend their cultivation to the more continental climate of Germany were of very limited success [12].

In the last decade, it became clear that winter hardiness of the winter bean material available at Goettingen (Goettingen Winter Bean Population 'GWP', see below) is more winter hardy than the UK and France cultivars [9]. Moreover, research on the reproductive mode of this material showed a cross-fertilization rate of about 60% [10], i.e., at least as high as earlier report for spring bean [13]. No experimental data are available on the agronomic performance of this material. Therefore, objective of the present study was to evaluate the agronomic performance

and the effect of selfing of this promising material as a prerequisite for further breeding activities.

## MATERIALS AND METHODS

The material used consisted of the Göttingen winter bean population 'GWP', a winter faba bean founded at Goettingen in 1989 (Stelling 1989, unpublished) by mixing 11 highly inbred winter bean lines of European gene pool (for details, cf. Gasim et al. 2004). Based on the harvest of 1999, three entries from this material were used, namely, (1) the open-pollinated (OP) version, considered as heterotic generation and (2) a once-selfed ( $S_1$ ) and (3) a twice-selfed ( $S_2$ ) versions, considered as inbred generations as well as four UK checks (Punch, Striker, Target and Clipper). These entries and the checks were assessed for agronomic performance and yield. The experiment was conducted for two consecutive seasons (2001 and 2002) at four environments at Göttingen, Germany. In the first season (2001), two entries were used, the open-pollinated GWP and  $S_1$ . The open-pollinated entry 2001 was formed by bulking equal amounts of seed from each individual of the population harvest of 1999. Similarly, the once-selfed entry 2001 ( $S_1$ '01) was produced by taking single seeds from 1999 population, growing them in isolation cages in 2000 as individual-plant single-seed-descend (IP'99-SSD), selfing plants and bulking equal amount of seeds per IP'99-SSD. The seeds of the entries and the checks were sown in autumn 2000 at two environments at Goettingen (Landwehr and Rennen). The sowing was in plots of 7.5 m<sup>2</sup> in a complete randomized block design with three replicates. Pertinent agronomic characters were assessed. Insecticides and fungicides were applied as required. In the next season (2002), three entries, open-pollinated, once-selfed and twice-selfed GWP were used as well as the four UK checks. The open-pollinated 2002 entry was formed by taking an equal amount of seeds from each individual of the population harvest of 2000 (Pop'00). Similar to the previous season, the once-selfed entry of the 2002 ( $S_1$ '02) was produced by taking single seeds from Pop'00, growing them in isolation cages as IP'00-SSD. The twice-selfed entry 2002 ( $S_2$ '02) was produced by taking single seeds from each  $S_1$ '00-SSD, selfing the plants and bulking equal amount of seeds per  $S_1$ '00-SSD. The seeds of the entries were sown at another two environments (Pfungstanger and Lachgraben) at Goettingen. Insecticides and fungicides were applied as required. The sowing was on October 23 for both seasons.

Throughout the environments, data from the following traits were recorded:

- (i) Winter damage, determined by score (1 to 9); 1=

Table1. Mean performance of some agronomic traits of the openpollinated GWP population and the UK checks (Punch, Striker, Target and Clipper) across four environments at Goettingen (Landwehr, Rennen in 2001, Pflingstanger, Lachgraben in 2002) in Germany

Traits						
Genotype	Winter damage (score)	Anthesis (days)	Plant height (cm)	Lodging (score)	TSW* (g)	Yield t/ha
GWP	1.7	133	144.6	7.7	615	5.40
Punch	2.4	133	137.6	6.8	621	5.50
Striker	2.5	135	144.5	7.0	632	5.56
Target	2.8	135	144.6	7.3	673	5.68
Clipper	2.6	136	140.4	7.3	676	5.71
<sup>1</sup> mean	2.6	135	141.8	7.1	651	5.61
LSD (0.05)	0.4	0.6	6.8	0.8	23	0.47

<sup>1</sup>mean of the checks (Punch, Striker, Target and Clipper)

\*TSW= Thousand seed weight

no winter damage, 9= complete damage,

(ii) Anthesis, determined by the number of days from first January to the day when 50% of a genotype opened the first flower,

(iii) Plant height measured in cm at the end of flowering,

(iv) Lodging, determined in a score (1 to 9); 1 fully upright, 9 totally lodged,

(v) Thousand seed weight (TSW), in grams, extrapolated from weighing 3x50 seed per plot, and

(vi) yield, measured in t/ha

The computer program PLABSTAT [23] was used for statistical analysis of the collected data. The data were analysed according to the complete randomized block design.

The mean performance of the open-pollinated version of the GWP and the mean performance of the inbred versions were used to estimate the effect of inbreeding depression due to selfing as follows:

$$\text{Inbreeding depression} = P_o - P_i$$

Where,  $P_o$  = Mean performance of the open-pollinated generation,

$P_i$  = Mean performance of an inbred generation (i= 1, 2;  $S_1$ '01,  $S_1$ '02, and  $S_2$ '02, respectively) for the specific traits.

Relative inbreeding depression was calculated as the ratio of absolute inbreeding depression to  $P_o$  values, and expressed as percent.

The expected inbreeding coefficient (F) realized in the open-pollinated generation was estimated based on the results of cross-fertilization rate (C) of the population (C = 60%, [10] and on Wright's equilibrium equation [24] as follows:

$$F = 2C/1+C.$$

The expected inbreeding coefficient realized in  $S_1$  and  $S_2$  were derived accordingly.

Yield and winter damage traits of these more or less inbred entries were regressed onto their expected inbreeding coefficient. The result was used to extrapolate the full heterotic difference between a fully outbreed and fully inbred version of this population, hence predict heterosis.

## RESULTS AND DISCUSSION

Analysis of variance (details not given) estimated for the open-pollinated GWP and  $S_1$  (as a group), open-pollinated GWP and the checks (as another group) revealed significant differences in winter damage (over wintering ability), anthesis, plant height, lodging and thousand seed weight. The effect of environment was significant for all

Table 2. Mean performance of some agronomic traits of open-pollinated (OP), once-selfed ( $S_1$ ) and twice-selfed ( $S_2$ ) generations of the GWP across four environments (Landwehr and Rennen in 2001, Pflingstanger and Lachgraben in 2002) in Germany.

Genotype	Traits					
	Winter damage (score)	Anthesis (days)	Plant height (cm)	Lodging (score)	*Tsw (g)	Yield (t/ha)
OP	1.7	133	144.5	7.7	615	5.40
$S_1$	2.6	133	130.5	7.8	592	4.57
$S_2$	3.1	134	128.6	8.3	591	3.38
<sup>1</sup> LSD (0.05)	0.5	0.5	7.1	0.8	22	0.45

<sup>1</sup>LSD does not apply for twice-selfed generation: data are from two environments in 2002 only (Pflingsanter and Lachgraben): twice selfed generation mean is adjusted for environment effects

\*Tsw = Thousand seed weight

these traits except for winter damage.

The mean performance of the open-pollinated GWP and the checks is given in table 1. The open-pollinated GWP showed better performance in over wintering ability than the checks. Field trials conducted at south Germany for effect of winter damage on the GWP using UK cultivars as checks showed the same result [9]. On the other hand, the GWP and the checks exhibited equivalent performance in plant height, lodging and yield. The results indicate the promising potential of the GWP as agriculturally competitive winter crop in Germany.

Data on the effect of inbreeding depression due to selfing on the performance of present-time European winter beans are few. In spring beans, a reduction in yield [20], plant height and thousand seed weight [8] were reported in the population deriving from selfing. For yield, a reduction of about 11% was reported [20]. In the present study, selfing was found to change the performance of  $S_1$  and  $S_2$  progressively away from the open-pollinated generation and the two levels of selfing caused clearly different effects, i.e., the performance of  $S_1$  and  $S_2$  show inbreeding depression to their open-pollinated population (table 2). Winter damage was smaller in the open-pollinated GWP (mean score was 1.7) than in once-selfed (mean score was 2.6) or in twice-selfed (mean score, corrected for environmental effect was 3.1) versions, i.e., the open pollinated version showed more resistance to winter damage than once-and twice-selfed versions.

For yield, the open pollinated GWP disclosed a reduction of 0.83 t/ha in  $S_1$  and a reduction of 2.01 t/ha in  $S_2$ ; the relative reduction was 15% in  $S_1$  and 37% in  $S_2$ .

Regarding plant height, the open-pollinated population showed a reduction of 14cm in  $S_1$  and 16cm in  $S_2$ , i.e. the relative reduction was 9% in  $S_1$  and 11% in  $S_2$ . Thousand seed weight showed a reduction of about 23 g in  $S_1$  and 24 g in  $S_2$ . This reduction in the performance of the GWP population due to selfing must be attributed to the highly heterogeneous and heterozygous nature of the faba bean population which renders it vulnerable to inbreeding depression enforced by selfing. The GWP was found to propagate with a cross-fertilization rate of on average of about 60%; so roughly about 60% of non-inbred  $F_1$  individuals may exist in the population [10] as well as further inbred individuals of different inbreeding status (cf. Link et al. [16]). It is thought that as evolutionary consequence of selfing, a high level of out-crossing would be associated with strong inbreeding depression and high self-fertilization with weak inbreeding depression.

The reduction in the above-mentioned traits in  $S_1$  and  $S_2$  generations of the GWP compared to its open-pollinated version verify the presence of heterosis in the open-pollinated generation. The extrapolated mid parent heterosis of about 32% for winter damage (Fig. 1) and 75% for grain yield (Fig. 2) was quantified for the open-pollinated version. These heterosis data are only a rough estimate, because (1) they are calculated from an approximate scale of  $0.19 < P < 0.76$  and extrapolated to  $0.0 < P < 1$  assuming a linear relationship, (2) one data point ( $S_2$  in 2001 environments) had to be estimated because it could not be taken from the experiment. The rather small but obvious heterosis for winter damage in the present study shows that there might be some

natural selection in favor of less inbred plants, hence, the inbreeding coefficient of a population after winter might be higher than the actual inbreeding coefficient due to natural selection for less inbred plants.

For yield, a value of  $R = 0.48$  showed that this approach most probably results for this trait. Similar amount of mid parent heterosis of about 75% in yield was reported for spring bean [5], [16]. From these results, it could be

concluded that the over wintering ability and yield exhibit a major impact of heterozygosity in winter beans.

**Application to breeding strategies**

Partially allogamous crops are bred as either self- or cross-fertilizers. Breeding as self-fertilized crop (line cultivar) leaves heterosis unexploited, moreover, there is a risk of lower yield stability [16], [21]. It is also expected that in winter bean, line cultivars may suffer from a loss of

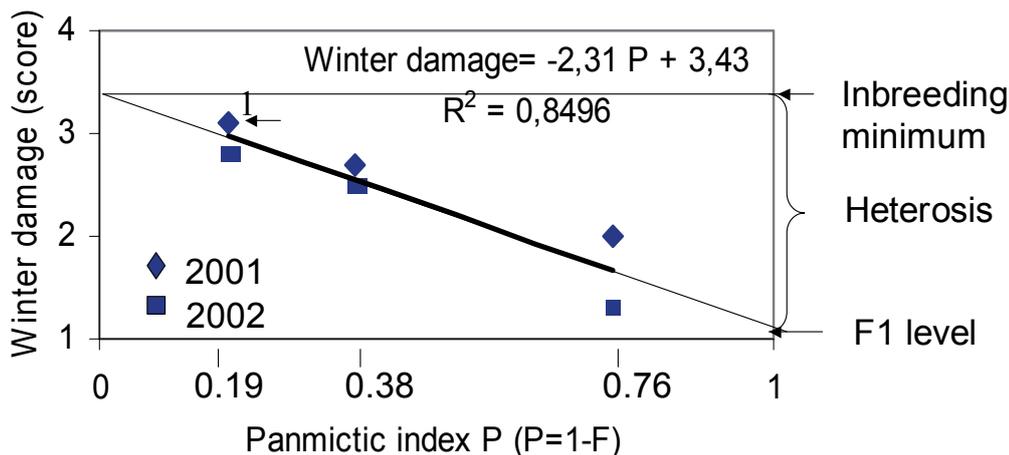


Figure 1. Mid parent heterosis in winter damage of the GWP winter damage as resulting from extrapolation (cf. text)

<sup>1</sup>The arrow points to the substitute  $S_2$  winter damage, estimated according to Healy and Westmacott [11] as implemented in PLABSTAT.

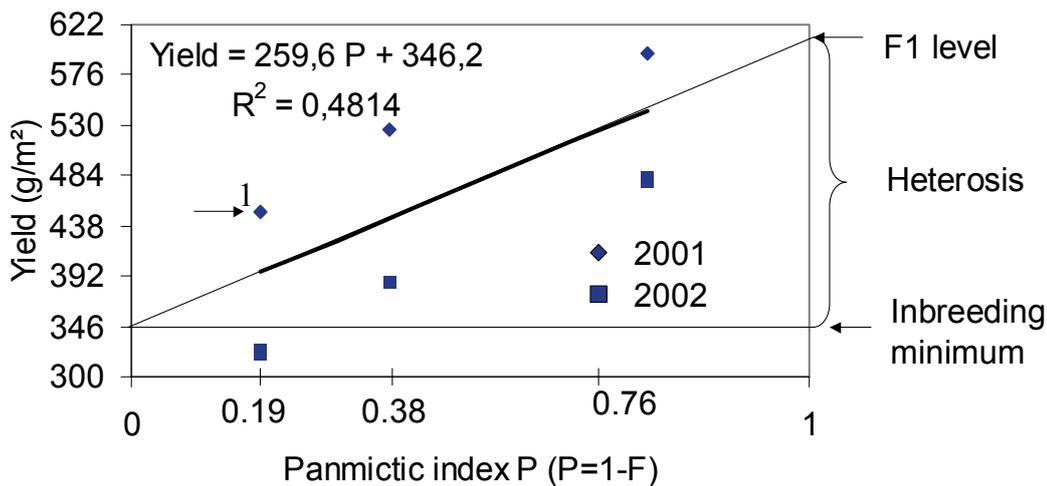


Figure 2. Midparent heterosis in yield of the GWP as resulting from extrapolation (cf. text).

<sup>1</sup>The arrow points to the substitute  $S_2$  GWP yield estimated according to Healy and Westmacott [11] as implemented in PLABSTAT

fitness referring to winter hardiness. Therefore, breeding line cultivars is of limited promise and not recommended as breeding strategy for the present material. Breeding as cross-fertilized crop makes hybrid cultivars an attractive possibility. Although more than 40 years after the first cytoplasmic male sterility (CMS) have been known in faba bean, no hybrid is produce, mainly because of the insufficient stability of the existing CMS system [3], [14].

To utilize part of the heterosis in faba bean, synthetic cultivars were recommended for spring beans [16] in Europe; the advantages of these cultivars are not only their partial use of heterosis, but also the possible increase in yield stability [21]. From the present study, synthetic cultivars bred from the GWP should realize about  $\frac{3}{4}$  of the total heterosis (75%) present in this material [15], [10]; this result is firmly in favour of synthetic cultivar to be developed from this material. The overall performance of the synthetics depend mainly on the number of parental components, their general combining ability, their specific combining ability and on the total amount of heterosis. Poly-cross and top-cross tests were used to identify parents with superior general combining ability. These methods are based on the assumption that the degree of cross-fertilization is equal for each parent; thus, differences in the performance of the progenies are thought to result from variation in the ability of maternal parents to transmit high performance to their progeny. However, in faba bean, this basic assumption may be partially invalidated. Link [13] and Maalouf et al. [18] pointed to an additional factor, different level of cross-fertilization, otherwise the combining ability estimates will include varying shares of per se performance. Variation in cross-fertilization among parental components could limit the efficiency of both top-cross and poly-cross progeny test to detect lines with superior general combining ability [13].

In addition to the methods used to evaluate the parents, several indices (e.g., Wright [24], Ederer and Link [6], Maalouf et al. [18]) have been developed to estimate synthetic value. Most of the indices used in the faba beans to identify the best parents for a synthetic variety fall into two broad categories: (1) the agronomic value of the parents and their crosses (assuming crosses occur at random), and (2) the parents' degree of cross-fertilization. For synthetics whose components are inbred lines, the optimum number of the parental components proved to be about six for European spring beans [6]. These numbers make reasonable use of the additive genetic variance in selection as well as providing the best balance between the danger of too high proportion of sibbing with few components and the inclusion of more lowly ranked

components if the number is greater [15].

For the present material, in addition to the presence of marked heterosis in yield and winter hardiness, previous research on this material showed: high cross-fertilization rate (60%),  $S_1$  lines with high heritability ( $h^2 = 0.75$ ) for this trait, and the ease of finding lines with cross-fertilization values of 60-70% [10]. Therefore, the prerequisite of breeding synthetic cultivars are present; but to put synthetic cultivars of the GWP in practical farming, further research is needed on:

- (1) breeding of lines with high cross-fertilization rate,
- (2) improvement of agronomic performance,
- (3) breeding of lines with increased overwintering ability,
- (4) breeding for lines with ascochyta resistance,
- (5) improvement of lines for standing ability and nutritional quality, and
- (6) increasing diversity and heterosis by an inclusion of genetically "distant" winter hardy bean line [19].

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