

SOME ABERRANT VARIANTS IN M₂ LINES AND THE RESULTING M₃ LINES OF NARROW-LEAFED LUPIN (*LUPINUS ANGUSTIFOLIUS*) ORIGINATING FROM EMS APPLICATION

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ABSTRACT

The treatment of 40,000 seeds of narrow-leaved sweet lupin (*Lupinus angustifolius*), cv. 'Boruta' with ethyl methanesulfonate (EMS) resulted in about 20,000 M₂ lines. A first series of 2,000 M₂ lines were grown in 2007 to be examined for aberrant plants. Several kinds of characters were considered, e.g. leaf shape, leaf colour, plant vigour, branching type, flower colour. Seeds were obtained from 1,438 aberrant plants displaying alterations in one to four visually assessed traits. Some combinations of altered traits coincided with altered seed numbers per plant. In 2008 666 M₃ lines were grown in the field to check heritability of the alterations observed in generation M₂. Of the M₃ lines, 520 were homogeneous for the mutated traits or trait combinations. Some of the lines are described and discussed regarding their potential for improving the agronomic value of narrow-leaved lupin as a crop.

KEYWORDS

ethyl methanesulfonate, EMS, seed treatment, mutation, *Lupinus angustifolius*

INTRODUCTION

The young history of the narrow leaved sweet lupin (*Lupinus angustifolius*) as a grain legume crop is particularly influenced by the discovery and utilisation of spontaneous mutations. These mutations concern typical wild type characters, e.g. high alkaloid content of seed and leaf, impermeable seed coat and seed and pod shattering. One of the pioneers of sweet-lupin breeding was Reinhard von Sengbusch who started 80 years ago a broad selection programme in several cultivated lupin species, to eliminate those wild type characters which seriously affected the commercial utilisation of this protein rich seed legume. Applying N.I. Vavilov's Law of Homologous Series in Hereditary Variation which had been phrased some years before, von Sengbusch and his co-workers, notably Hackbarth, Troll and Zimmermann, examined about 3-4 millions of plants from *L. albus*, *L. angustifolius* and *L. luteus* for the occurrence of natural mutants with decreased alkaloid content, improved resistance to seed and pod

shattering, low seed dormancy, and permeable seed coat, respectively. One of the most important results was the discovery of two plants out of 370,000 screened *L. angustifolius* plants with low alkaloid contents. That was the starting point for the creation of a new agricultural crop: the sweet lupin.

The adaptation of grain lupines to the challenges of modern agriculture needs varieties with increased agricultural benefits. In this context the enhancement of genetic variability for the selection of desired variants is of increasing importance for the breeding of varieties for new growing areas or organic farming (Gladstones *et al.* 1998, Kurlovich, 2002). One important way to achieve this objective is the use of induced mutations. Therefore a mutation programme was started in 2006 with the German sweet lupin cv. 'Boruta'. In the present report we describe the genetic variability induced by EMS mutagenesis and observed in the M₃ generation.

MATERIALS AND METHODS

Mutagenic treatment was applied to cv. 'Boruta' (breeder: Saatzucht Steinach GmbH), a determined type with white seeds and a 1000 seed weight of 148 g.

A solution of 1.75% ethyl methanesulfonate (EMS; Sigma-Aldrich® Germany) was applied. After soaking the seeds with water, they were submerged for four hours in the EMS solution. Subsequently, the seeds were decontaminated with 250 mM sodium thiosulfate, rinsed with tap water, and dried back for sowing. The dried seeds were sown with a plot driller in a large plot of 40 m x 12 m with a seed density of 83 seeds/m². Mature M₁ seeds were harvested separately for each plant, with each offspring forming a separate M₂ line.

RESULTS AND DISCUSSION

M₂ LINES

The dried M₁ seeds were sown on 13 April 2006. As expected the emerging plants were heterogeneous in early development as well as in flowering and maturing. Mature M₁ plants were harvested individually at several times. Many plants showed strongly delayed seed set or lacked seed set. The M₂ offspring were classified

according to their seed set. Of the 27,301 M₂ lines about 70 % had fewer than 20 seeds (Fig. 1).

In 2007 a first set of 2,016 M₂ lines was grown in the field. Twenty seeds per line were sown with a single seed driller on 27 March in a 3 m long row (plant distance of 15 cm). Seedling emergence and early development were disturbed by heavy rainfall after sowing. In repeated screenings during the vegetation period the plants of each line were examined individually for several traits and aberrant plants were labelled for harvesting. Traits assessed were: leaf shape, leaf colour, plant vigour, branching and determination, and flower colour. In most cases different aberrant types

Table 1. Numbers of M₂ plants recorded for single altered traits and combinations thereof.

No. of altered traits/plant	No. of plants	Percentage (%)	No. of seeds/plant
1	685	48	36
2	401	28	44
3	294	20	74
4	58	4	109
Total	1,438		

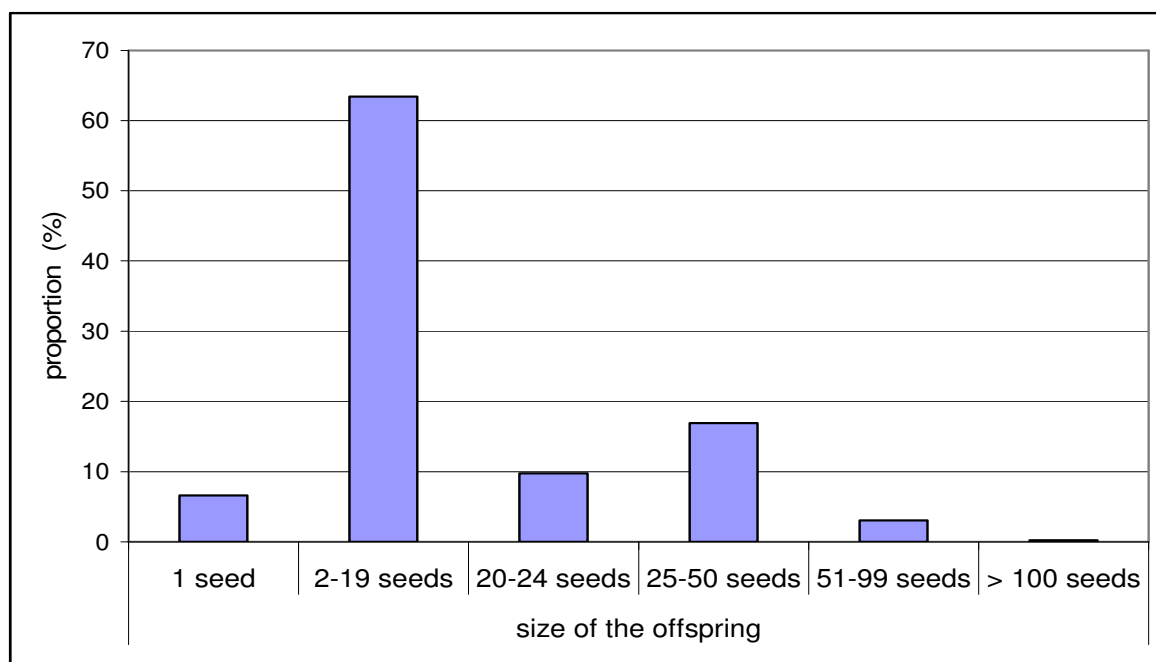


Fig. 1. Distribution of M₂ line size harvested from M₁ plants in 2006.

were identified for the respective trait, namely for leaf shape: 'broad-leafed' (bl) and 'narrow-leafed' (nl); for leaf colour: 'dark-green leaf' (b), 'yellow-green' (v), 'lemon-yellow' (c), 'pale' (p); for vigour: 'very vigorous' (xl), 'weak' (w), 'subtle' (sb), 'semi dwarf' (dws), 'dwarf' (dw); for branching and determination: 'zero branched' (zb) with no branches and 'multi-branched' (mb), 'highly branched' (hb) with a non-branched stem base but fertile branches in the upper part of the stem, a multi-branched, indeterminate type with flowering branches above the main inflorescence (vnd), and 'non reduced' (nr) with non reduced pod set on the main stem with pod distribution over the whole inflorescence. The focus was on traits which are thought favourable for plant growth and fertility. Plants with aberrant leaflets and stems, which died off very early, were ignored in this paper. In the most cases aberrant plants were altered in one trait. Plants with one to four altered traits occurred with decreasing frequencies (Table 1). A number of plants carried empty pods. Of

the 1,438 M₂ plants harvested 1,157 yielded at least one kernel.

The number of seeds recorded per plant was not exact in every case; nevertheless it provided information about the coincidence of a certain trait with fertility. Fig. 2 shows the mean seed yields of individuals displaying a single altered trait. Only one plant was found that carried 'xl' as a single altered trait.

Different combinations of altered traits coincided with largely differing seed yields, with the highest seed yield (196 seeds/plant) observed for the combination of the four traits 'b'+ 'b'+ 'mb'+ 'hb' (Fig. 3). The mean seed set of the check cv. 'Boruta' was 164 seeds/plant. As expected, dwarf (dw) or semi-dwarf (dws) types displayed reduced seed yields. Trait combinations involving the altered trait 'nr' displayed pods with only few seeds in several cases. The observed variability in 'nr' could permit selection for pod filling in these variants.

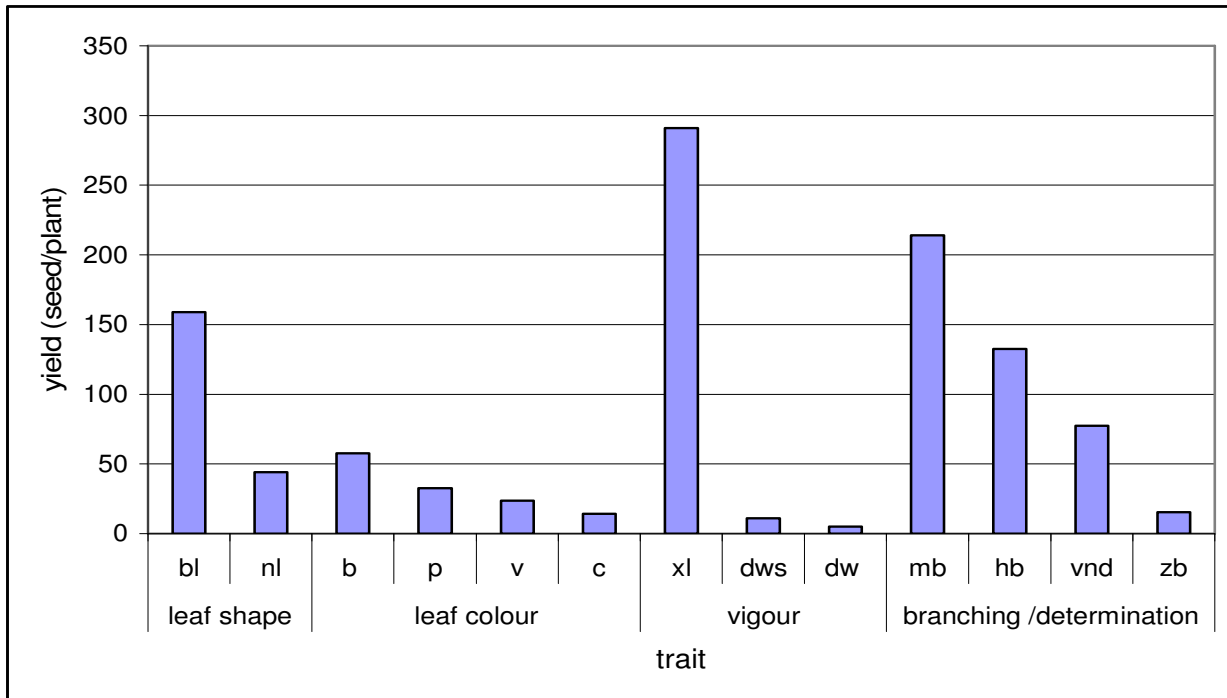


Fig. 2. Mean yields (seeds/plant) of plants characterised by a single altered trait.

These results suggest that certain favourable variants may be used for variety improvement. However, they need to be genetically stable M_3 lines or beyond and their inheritance has to be studied.

M_3 LINES

In 2008, 666 M_3 lines were sown in one row (20 plants per line) or two rows (40 plants per line) in the same manner as the M_2 lines in 2007. Most of the lines were homogeneous, whereas the variation between lines was considerable. In several cases the phenotype of the M_2 seed parent was not confirmed in M_3 , suggesting an environmental rather than a mutational background of the respective phenotype. For instance, in one case the M_3 line from a semi-dwarf ('dws') M_2 plant was not semi-dwarf, in other cases the M_3 lines from yellow-green ('v') M_2 plants displayed a normal leaf colour.

Among the homogeneous M_3 lines, some had interesting phenotypes. Two lines displayed broad leaves, one of them with two to three carpels in one flower; other lines were characterised by short stems and very narrow leaves of deeply dark-green colour, showing the same growth habit as their M_2 parents ('dw' type). Several lines displayed a bushy growth habit with many branches; others had a considerably reduced number of branches. One line did not develop a main stem, but only basal branches. Another line was of a fasciculate type with a main stem which divided in two or three inflorescences.

The observation of the M_3 lines will be continued over the whole growing season concerning several pod characters as well as pod density and plant yield. The observed phenotypic variability as compared to the untreated cv. 'Boruta', which was grown together with the M_3 lines, gave a strong indication for successful mutagenesis via EMS treatment. We expect that EMS will have some effects on seed and plant composition as well. Analyses of seed protein contents and amino acid composition will be done after the harvest of the M_3 lines. A separate mutagenesis study with M_2 plants concerned the improvement of the tolerance to soluble lime in the soil. This might improve the agronomic value of the crop by extending its area to the alkaline soils. Currently, the growing of narrow-leaved lupin has been restricted to slightly acidic soils.

The mutagenesis programme will be continued with the examination of further sets of M_2 lines in 2008 and 2009.

The described results suggest that EMS treatment of narrow-leaved lupin (*L. angustifolius*) is able to induce genetic variability in this crop plant to a considerable extent. The great number of homogeneous M_3 lines obtained from individual M_2 plants indicates the occurrence of heritable changes due to the mutagenic treatment. The amount of 40,000 EMS-treated seeds should provide a sufficient base for the selection of favourable mutants which can be used for the further improvement of the crop.

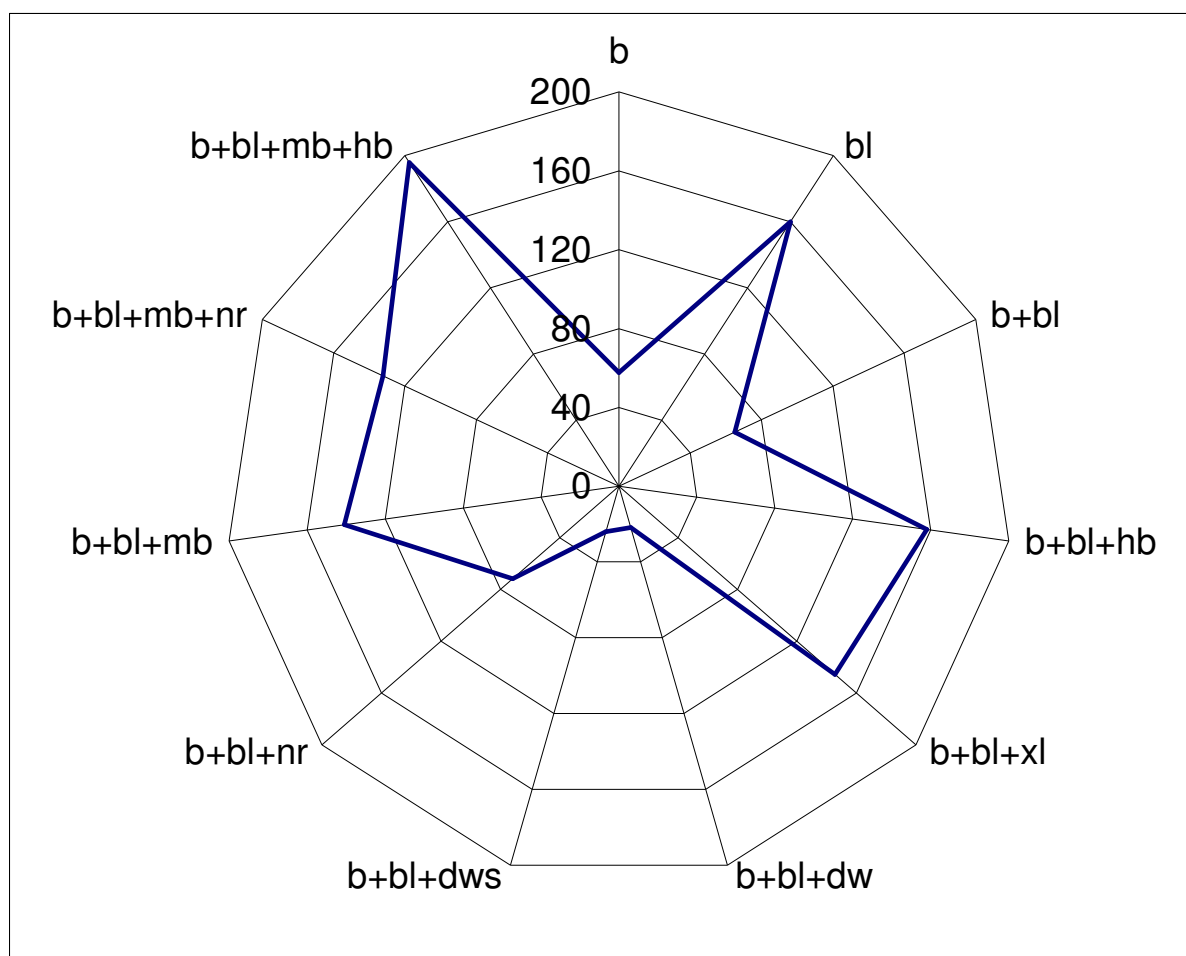


Fig. 3. Mean seed yields of aberrant phenotypes displaying single traits 'b' and 'bl' and their combinations with other altered traits (diagram axis: number of seeds/plant).

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